

Japan-U.S. BSE discussion WG

Ministry of Health, Labour and Welfare

1. Definition of BSE and its testing methods

(1) Testing methods

① Screening

‘Platelia’ ELISA-kit (Bio-rad Laboratories), Enfer BSE test (Enfer) (Appendix 1)

② Confirmation

Western Blot, Immunohistochemical examination (Appendix 2)

③ Ground law and testing system in abattoirs

- Ground law

Based on Article 14 of the Abattoirs Law, only animals that pass the ante-mortem and post-mortem inspection that are conducted by meat inspectors (all are veterinarians) who are public officials of prefectures or cities establishing health centers and are approved for the slaughter and dressing for use as edible meat (Appendix 3). It is designated that cattle of 0 months or older (all ages) are subjected to BSE testing in this inspection based on the Law Concerning Special Measures for Bovine Spongiform Encephalopathy (Appendix 4).

The Abattoirs Law prohibits producing meat from cattle affected with BSE, and cattle diagnosed with BSE are incinerated and the processing facilities are disinfected.

- Testing system

On October 18, 2001, BSE testing was introduced in all meat inspection in all abattoirs where cattle are slaughtered.

For cattle suspected of having BSE in ante-mortem inspection with neurological symptoms, etc., slaughter is prohibited. In addition, if an animal tests positive in a BSE screening test in post-mortem inspection, confirmation testing is conducted at the National Institute of Infectious Diseases, Obihiro University of Agriculture and Veterinary Medicine, or Hokkaido University and determinative diagnosis is given by the “Expert Committee for BSE Diagnosis, MHLW” based

on the test results (Appendix 5).

Testing has been conducted on 3,159,408 animals as of May 8, 2004 (Appendix 6).

There are 162 abattoirs where cattle are slaughtered and dressed (as of February 2004) and 2,657 meat inspectors (as of March 31, 2003).

(2) Definition

① Process of diagnosis and BSE cases in Japan (including atypical cases)

Diagnosis in BSE testing based on the Abattoirs Law is implemented by the “Expert Committee for BSE Diagnosis, MHLW” established in the Ministry of Health, Labour and Welfare, and positive cases from the BSE screening test are diagnosed conclusively based on the results of confirmation tests.

The criteria for diagnosis as BSE consist of a positive result in either Western Blot or Immunohistochemical examination in the confirmation test. Two of the 11 cases diagnosed as BSE in Japan so far have had positive results only in Western Blot, and the results of Immunohistochemical examination were negative. Furthermore, histopathological tests are also conducted in the confirmation test, and 5 animals among the 11 diagnosed with BSE did not show spongiform symptoms in their brain tissues (Appendix 7).

Since a small amount of PrP^{Sc} with an electrophoretic profile different from that of typical BSE-associated PrP^{Sc} was seen in Western Blot testing for cattle slaughtered on October 18, 2003, the results were published (Appendix 8) and information was provided to the OIE. This case was diagnosed as bovine prion disease or BSE because abnormal prion protein was confirmed in Western Blot testing.

② Process of diagnosis in EU

The EU also considers positive results in any of immunohistochemistry, immuno-blotting, and the demonstration of characteristic fibrils by electron microscopy as BSE positive based on the TSE regulation (Appendix 9).

(3) Tests on sheep and goats

Since the possibilities of BSE being transmitted to sheep and goats cannot be denied, Western Blot testing is implemented on all sheep and goat animals of 12 months or older that are to be supplied as meat in a similar fashion to BSE based on the Abattoirs Law. A total of 529 animals have been tested since May 2001 and no TSE cases are confirmed.

2. Definition of SRM and method of removal

(1) Ground law

Based on Article 6 of the Abattoirs Law and Section 2, Article 7 of the Law Concerning Special Measures for Bovine Spongiform Encephalopathy, owners or managers of abattoirs are required to contain bovine heads (except for tongues and cheek meat), spinal cords and distal ileum (2 meters from connection to caecum) in a special waste container for incineration (Appendix 3, 4).

Similarly, based on Article 9 of the Abattoirs Law and Section 3, Article 7 of the Law Concerning Special Measures for Bovine Spongiform Encephalopathy, slaughter businesses have been required to process bovine heads (except for tongues and cheek meat), spinal cords and distal ileum (2 meters from connection to caecum) so that contamination of the dressed carcass and edible intestines is prevented since October 18, 2001, and related documents have been provided by the Ministry of Health, Labour and Welfare (Appendix 10).

On the other hand, the use of the vertebral column for food by meat processing and other food businesses has been prohibited based on Section 1, Article 11 of the Food Sanitation Law since February 16, 2004.

Brains, eyes, spinal cords and placentas of sheep and goat aged over 12 months, and tonsils, spleens and large and small intestine of those of all aged have been removed and incinerated since April 1, 2002.

(2) Method of removal/incineration and supervising system

In abattoirs, the removal, disposal and incineration of specified risk materials are implemented under the supervision of meat inspectors who are public officials of prefectures, etc. In addition, it is also accepted that licensed industrial waste processing businesses must incinerate outside the properties of abattoirs (Appendix

12).

For meat processing facilities and butcher's shops, food inspectors of prefectures, etc. have witness inspections regularly to confirm observance.

Procedure for Bovine Spongiform Encephalopathy (BSE) Screening Test

No.1: Treatment of prion materials in laboratories

- Work shall be conducted inside the safety cabinet in a special compartmented laboratory in principle.
- In order to prevent infection from cuts and contamination of eyes and mouths by droplets, workers shall wear latex or vinyl gloves, masks, preventive clothes and caps as well as protective glasses, etc. if necessary.
- Disposable working clothes, tools and equipment shall be used as much as possible.
- Test material shall be handled on a bench seat with caution not to generate droplets and aerosol.
- When the test material is spilled and when work has been completed, the surface of the worktable shall be cleaned with sodium hypochlorite solution.
- The bench seat used, disposable tools, etc. shall be placed in an autoclave bag for autoclave sterilization at 132 – 134°C for 60 minutes.
- Since scissors, tweezers, etc. are reused, wipe off the stains with tissue, etc. or cotton soaked in alcohol, and soak in 3 – 5% SDS to boil for 5 – 10 minutes (addition of sodium carbonate to 1% will prevent metal corrosion) or sterilize in an autoclave at 132 – 134°C for 60 minutes.
- Plastic tools that cannot be heated shall be soaked in sodium hypochlorite at 5% or higher or NaOH of normality 2 or higher for 2 hours or longer.
- Inflammables shall be put into an autoclave bag for autoclave sterilization at 132 – 134°C for 60 minutes.
- If an incinerator for medical waste is available, plastic tools and inflammables shall be placed in a biohazard bag together to be incinerated.
- If the outer surface of the centrifuge tube, etc. which needs to be kept operating, becomes contaminated, replace with an uncontaminated before continuing working.
- If only a normal autoclave is available, it is also valid to put the contaminated objects in an alkali-resistant container and soak in NaOH of normality 1 – 2 at 120°C for 30 minutes.
- When decontaminating tools, equipment, etc. that cannot be incinerated, wipe off with inflammables such as paper and cotton soaked in alcohol first and then conduct the sterilization process in principle since the amount of remaining prion will be large as a matter of course for highly contaminated cases and pieces of tissue, etc.

No.2: Collected parts

After separating the head from the dotted line in Figure 1, insert a spoon from the osculum and collect the specimen so that the Obex (shaded area in Figure 1) shown in Figures 1 and 2 is included. The shaded sections in black indicate the skull and cervical vertebra. Since lesion and accumulation of prions occur nearly symmetrically, divide into 2 at the mesial line to fix one with formalin buffer of 15 – 20% concentration as material for the histopathological test and the immunohistochemical test, and use the other as the material for the immunobiochemical test (ELISA method, western blot method, etc.).

Use a tissue flake from the immunobiochemical test material that cuts across so that Obex is included as the screening test specimen. Since prion is not always accumulated uniformly inside the Obex, it is necessary that the specimen be collected uniformly. Hence, the cut tissue flake shall be cut into small pieces with scissors to be mixed and balanced for fractionation of the necessary volume in preparing the specimen. However, this does not always apply if the specimen is collected according to methods other than cutting.

No.3: Procedure for using the bovine spongiform encephalopathy kit

Screening test shall be conducted using “Platelia BSE” or “Dynabott Enfer BSE” testing kit, and the procedure for using each is as provided in Appendixes 1-1 and 1-2.

No.4: Storage of test results

The raw data read by the micro plate reader shall be input in separate Form 1-1. Input the other items to be input on separate Form 1-1 and store the signed paper as well as its corresponding electronic data. In addition, affix the raw data read by the micro plate reader to the paper to be stored (if it uses sensitive paper, print it on normal paper). When sending the specimen for the confirmatory test, a copy of the stored data affixed with raw data shall be sent along.

No.5: Sending the specimen for the confirmatory test

For specimens judged positive¹ using the bovine spongiform encephalopathy kit, specimens shall be sent for the confirmatory test according to the following method except for cases in which the confirmatory test is implemented by the Prefecture, etc.:

1. Destination

Destination shall be as provided in the attachment to the notification No. 0407001 issued by the Inspection and Safety Division dated on April 7, 2004.

2. Method of communication for the confirmatory test results

It shall be informed to the municipality requesting the test by the Inspection and Safety Division.

Furthermore, the contact for questions on this issue is the Milk and Meat Safety Section, Inspection and Safety Division (Phone) 03-3595-2337.

3. Sent sections (as shown in Figure 2)

Divide the tissue including Obex at the mesial line, and

- (1) Fix one with formalin buffer of 15 – 20% concentration and send at room temperature as material for the histopathological test and the immunohistochemical test. The size of the container for the specimen shall be 50 ml, and it shall be filled with formalin buffer.
- (2) To use the other as the material for the immunobiochemical test (ELISA method, western blot method, etc.), send it frozen. Remaining sample from the specimen collection and ELISA method (homogenized emulsion sample, etc.) shall also be sent along frozen.

4. Method of communication in sending

When sending a specimen judged positive in the screening test, attach the test results in separate Form 1-1 and the specimen sending form in separate Form 1-2 and send with specified date and time of arrival (morning: 9:00 – 12:00 or afternoon: 13:00 – 16:00) to the testing institute.

Furthermore, send the test results in separate Form 1-1 and specimen sending form in separate Form 1-2 to the Inspection and Safety Division (FAX) 03-3503-7964 and call the section (phone) 03-3595-2337 in advance.

Emergency contact on holiday, etc. will be notified later.

5. Precautions in sending the specimen

Based on No.2 and No.3, Article 8 of the Postal Regulations (Ordinance of the Ministry of Communications, No.34, 1947), containers of United Nations standard shall be packed, etc. appropriately for sending.

Furthermore, the pickup and delivery post office that takes charge of the address (referred to as “the post office in charge” hereafter) shall provide information on the method of transporting the corresponding package .

¹ Refers to the case in which the result of re-testing in No.3 is concluded as positive.

- (1) Package containing a specimen that is not transported by aircraft in the process of transporting

1) All necessary items shall be entered in the paper in the following form to be affixed on the package surface where it is easy to see.

<p>Article name: Bovine tissue, etc. "Hazardous matter" (Note 1)</p> <p>Sender:</p> <p> Municipality name:</p> <p> Test facility name:</p> <p> Address:</p> <p> Phone number:</p> <p> Qualification: Slaughter test personnel (veterinarian)</p> <p> Full name:</p>

(Note 1) To be written in red.

- (2) Package containing a specimen that is transported by aircraft in the process of transporting (Note 3)

1) All necessary items shall be entered in the paper in the following form to be affixed on the package surface where it is easy to see.

<p>Article name: Bovine tissue, etc. "Hazardous matter" (Note 1)</p> <p>UN Number:</p> <p>Sender:</p> <p> Municipality name:</p> <p> Test facility name:</p> <p> Address:</p> <p> Phone number:</p> <p> Qualification: Slaughter test personnel (veterinarian)</p> <p> Full name:</p> <p>Dry ice xxx kg contained (Note 2)</p>
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(Note 1) To be written in red.

(Note 2) To be written in red when sending with dry ice contained.

- 2) The container for specimen shall be a "container of United Nations standard."

- 3) Volume for 1 container shall be less than 1,000 ml for liquids and 50 g or less for solids.
- 4) Transport permissible object indication label (Class no.: 6.2) shall be affixed on the package surface where it is easy to see. (Note 4)
- 5) When dry ice is outside the United Nations standard container in cardboard package, etc., transport permissible object indication label (Class no.: 9) shall be affixed on the package surface where it is easy to see. (Note 4)
- 6) In the above case e, the post office personnel may request to open the cardboard box, etc. upon receiving the package to check if the specimen is contained in a United Nations standard container. This shall be granted.
- 7) Two copies of the hazardous matter application document shall be prepared to be submitted with the package. (Note 5)
Furthermore, an open envelope stating "containing hazardous matter application" shall be fixed to the package. The post office will check the information on hazardous matter application and return the document. Seal it in the corresponding envelope under the inspection of the post office personnel.

(Note 3) When transporting on aircraft, it is regulated by Article 86 of the Aviation Law, Article 194 of Aviation Law Enforcement Regulations and related notifications, etc.

(Note 4) The form of indication label shall be as shown in separate Form 1-3 (request the necessary quantity to the post office in charge).

(Note 5) The hazardous matter application document shall be as shown in separate Form 1-4 (furthermore, this is stipulated specially between the Postal Service Agency and airlines for transporting the specimens for this case, and it cannot be applied to others).

(Separate Form 1-1)

(Test / Re-test)

Test date: Year month day

Municipality name:

Test institute name:

Inspector name (signature):

Cutoff value:

-10% of the cutoff value:

Number of specimens:

(Number of positive/negative specimens)

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

	← Enter the sample name (code, etc. used for individual identification).
	← Enter the measurement value.

※1: The above table indicates the 96-hole plate. Enter values with correspondence to the wells used in actual testing.

※2: Draw a slant line on each unused well.

Appendix 1
(Separate Form 1-2)

Name of institute conducting confirmatory test:
Name of receiver:

Municipality name:
Person in charge:
Phone:

Bovine Spongiform Encephalopathy Confirmatory test Specimen Sending Form

1	Date sent	Source sending the specimen (name of test center)	Specimen no.	Specimen weight (g)	Date of specimen collection	Sex	Breed	Age in months	Clinical symptoms	Date of slaughter	Remarks
	Shipped by		Raised by								
	Name	Address	Phone	Name	Address	Phone					
2	Date sent	Source sending the specimen (name of test center)	Specimen no.	Specimen weight (g)	Date of specimen collection	Sex	Breed	Age in months	Clinical symptoms	Date of slaughter	Remarks
	Shipped by		Raised by								
	Name	Address	Phone	Name	Address	Phone					
3	Date sent	Source sending the specimen (name of test center)	Specimen no.	Specimen weight (g)	Date of specimen collection	Sex	Breed	Age in months	Clinical symptoms	Date of slaughter	Remarks
	Shipped by		Raised by								
	Name	Address	Phone	Name	Address	Phone					

Planned date and time of arrival at the test institute

(Separate Form 1-3)

1. Transport permissible object indication label (Class no.: 6.2)

INFECTIOUS SUBSTANCE
IN CASE OF DAMAGE OR LEAKAGE
IMMEDIATELY NOTIFY
PUBLIC HEALTH AUTHORITY

2. Transport permissible object indication label (Class no.: 9)

MISCELLANEOUS

(Separate Form 1-4)

(Transport by air)

Application on Hazardous Substance Contained in Mail (Bovine Tissue, etc.)

The article name, quantity, etc. for the postal package below are all precise, and they are contained in a United Nations standard container with packing, labeling, etc. according to the Aviation Law and its related regulations. This postal package falls within the range of the loading limit for aircrafts and it is in an appropriate condition for transport by aircraft.

Date of preparation of application		Year month day
Article name		Bovine tissue, etc.
UN2814 UN2900	Substances (liquid) that may transmit disease that is transmissible to humans and animals	(Note 1) ml
UN2814 UN2900	Substances (solid) that may transmit disease that is transmissible to humans and animals	(Note 2) g
UN1845	Dry ice	Kg
Dry ice is put outside the United Nations standard container to be packed in another container, etc.		

Shipped by
 Municipality name:
 Test institute name:
 Address:
 Phone number:
 Name: Slaughter inspector (veterinarian)

Received by
 Institute name:
 Address:
 Phone number:
 Name:

Space for use by airline company

(Note 1) If the contained substance is liquid, the total volume that can be put inside one container is less than 1,000 ml.

(Note 2) If the contained substance is solid, the total weight that can be put inside one container is up to 50 g.

(Separate Form 1-4) (Sample)

(Transport by air)

Application on Hazardous Substance Contained in Mail (Bovine Tissue, etc.)

The article name, quantity, etc. for the postal package below are all precise, and they are contained in a United Nations standard container with packing, labeling, etc. according to the Aviation Law and its related regulations. This postal package falls within the range of the loading limit for aircrafts and it is in an appropriate condition for transport by aircraft.

Date of preparation of application		October 30, 2002	
Article name		Bovine tissue, etc.	
	UN2814 UN2900	Substances (liquid) that may transmit disease that is transmissible to humans and animals	(Note 1) ml
✓	UN2814 UN2900	Substances (solid) that may transmit disease that is transmissible to humans and animals	(Note 2) 40 g
✓	UN1845	Dry ice	3 kg
✓	Dry ice is put outside the United Nations standard container to be packed in another container, etc.		

Shipped by

Municipality name: xxx prefecture
 Test institute name: yyy meat sanitation test institute
 Address: 1-2-3 zzz, aaa city
 Phone number: xxxx-xxx-xxxx
 Name: Slaughter inspector (veterinarian)
 xx xx

Received by

Institute name: yy Test Center
 Address: 〒000-0000 3-2-1 bbb city, ccc prefecture
 Phone number: xxxx-xxx-xxxx
 Name: xx xx

Space for use by airline company

(Note 1) If the contained substance is liquid, the total volume that can be put inside one container is less than 1,000 ml.

(Note 2) If the contained substance is solid, the total weight that can be put inside one container is up to 50 g.

(Reference Material)

Process for deactivating abnormal prion proteins completely

Table 1. Process for deactivating abnormal prion proteins completely²

Drug	Concentration	Process period	Temperature
Formic acid	≥ 60%	2 hours	Room temperature
Guanidine thiocyanate	≥ 4M	2 hours	Room temperature
Guanidine hydrochloride	≥ 7M	2 hours	Room temperature
Trichloroacetic acid	≥ 3M	2 hours	Room temperature
SDS	≥ 3%	5 minutes	100°C
Phenol	≥ 50%	2 hours	Room temperature

Table 2. Sterilization method for contaminated materials³

Drug, method, etc.	Temperature (°C)	Period (min)	Subject
Incineration	≥ 800	-	Viscera, inflammables, etc.
Autoclaving	134	60	Various equipment, devices, viscera, etc.
Soaking in 3% SDS	100	5	Various equipment, devices, etc.
Soaking in normality 2 NaOH	Room temperature	60	Various equipment, devices, etc.
Soaking in normality 1 NaOH	Room temperature	120	Various equipment, devices, etc.
Soaking in 1 – 5% sodium hypochlorite	Room temperature	120	Various equipment, devices, etc.

Cases should be dissected on a vinyl sheet spread out in an anatomy room, etc. and dissection should be limited to the minimum level necessary. When removing the head, contamination should be minimized by receiving the blood in a container. Take measures to place the separated head in a plastic bag and cover the cervical section with a plastic bag, etc. to prevent the spread of contamination.

²: Onodera Takashi, Kitamoto Tetsuyuki, Kurata Takeshi, Sato Takeshi, Tateishi Jun; Manual for Creutzfeldt-Jakob disease diagnosis, (edited by Disease Control Division, Health Service Bureau, Ministry of Health and Welfare), 18- 23, Shinkikaku Shuppannsya, Co., Ltd., Tokyo (1997).

³. Same as the above 2.

(Appendix 1-1)

Procedure for Using “Platelia BSE”**1. Sample refinement****(1) Preparation of reagents**

To meet the number of specimens to be processed (see the table below), dilute proteinase K to 250-fold with the dissolving solution (Reagent A) for BSE refinement kit.

(The diluted proteinase K solution can be stored at room temperature for 4 hours.)

Number of specimens	Reagent A volume	Proteinase K volume
2	2 ml	8 μ l
10	6 ml	24 μ l
18	10 ml	40 μ l
26	14 ml	56 μ l
34	18 ml	72 μ l
42	22 ml	88 μ l
50	26 ml	104 μ l
58	30 ml	120 μ l
66	34 ml	136 μ l
74	38 ml	152 μ l
82	42 ml	168 μ l
90	46 ml	184 μ l

(2) Refinement of abnormal prion peptides

- 1) Measure and take 350 ± 40 mg of the Obex section of the cattle.
- 2) Insert the measured specimen into a grinding tube.
- 3) Completely homogenize the sample in the grinding tube (the homogenized sample at this point can be stored for several weeks at -20°C . When storing the sample, freezing and defrosting shall be conducted only once).
- 4) Take 500 μ l and pour into a 2 ml microtube, etc. with caution not to contain solids from the homogenized sample (the sample can be stored for 8 hours at $2 - 8^{\circ}\text{C}$ and several weeks at -20°C).

- 5) Add 500µl of the proteinase K solution diluted in above 1. (1) and mix well. To unify the enzyme activation, add proteinase K solution and mix promptly within 5 minutes, or within 10 minutes when it is placed on ice.
- 6) When mixed well, promptly place in water bath, incubator, heat block, etc. for incubation at $37 \pm 1^\circ\text{C}$ for 10 ± 1 minutes. The period left between procedures 5) and 6) should not exceed 2 minutes.
- 7) Within 2 minutes after completion of incubation (or 10 minutes when tube is placed on ice), add 500µl of Reagent B and mix well until the entire solution turns blue (add Reagent B and mix promptly within 5 minutes, or within 10 minutes when it is placed on ice).
- 8) Centrifuge for 5 minutes at 20,000 x g or 7 minutes at 15,000 x g.
- 9) When centrifugal separation is finished, discard the supernatant within 5 minutes. To remove as much supernatant as possible, set the tube upside down and place it over paper for 5 minutes or suction dry with aspirator for 5 minutes.
- 10) When supernatant is discarded, add 50µl Reagent C1 to the microtube within 10 minutes. This must not be mixed using a vortex.
- 11) Promptly place in water bath, incubator, heat block, etc. for incubation at $100 \pm 1^\circ\text{C}$ for 5 ± 1 minutes. The period left between procedures 10) and 11) should not exceed 2 minutes.
- 12) Take out the microtube from incubator and mix well using a vortex (the sample can be stored for 5 hours at $2 - 8^\circ\text{C}$ or several weeks at -20°C at this point. When stored in either way, incubate at $100 \pm 1^\circ\text{C}$ for 5 ± 1 minutes and then mix well using a vortex).
- 13) Add 250µl of the diluting solution (R6) for BSE detection kit and mix (the sample can be stored for 5 hours at $2 - 8^\circ\text{C}$ at this point. When stored, mix well before moving on to the next procedure). When mixed, fractionate the sample to the well of detection kit microplate (continued to 2. (2) 2)).

2. Sample detection

(1) Preparation of reagents

- 1) The reagents to be used and microplate for solid-phasing shall be taken out of the refrigerator to be set to room temperature ($20 \pm 5^\circ\text{C}$) before use.
- 2) Dilute the undiluted washing solution (R2) with purified water to 10-fold and mix well to prepare the washing solution (R2') (this can be stored at $2 - 8^\circ\text{C}$ for 2 weeks).

- 3) Lightly tap and open the bottle of positive control (R4) to add 2 ml purified water or dilution solution (R6). Leave for 1 minute and mix slowly to dissolve (this can be stored for 2 hours at 2 – 8°C or 6 months at -20°C after fractionating to appropriate volumes). When storing by freezing, subdivide into microtubes, etc. immediately after defrosting and store at -20°C.
 - 4) Dilute the enzyme labeled antibody (R7) with the washing solution to 10-fold immediately before use and mix slowly to prepare the enzyme labeled antibody solution (R7'). One milliliter of enzyme labeled antibody solution (R7') will be required for each strip (this can be stored for 6 hours at 2 – 8°C).
 - 5) Mix the substrate buffer (R8) and coloring solution (R9) at the ratio of 10:1 in a light-tight container shielded with aluminum foil, etc. to prepare the substrate coloring solution (R8 + R9). One milliliter of substrate coloring solution (R8 + R9) will be required for each strip (this can be stored for 6 hours at room temperature. However, it cannot be used if it has turned blue before use. Prepare a new solution again in such cases.)
- (2) Detection of abnormal prions
- 1) Take out the required number of strips from the microplate rack (return the strips that are not to be used into the bag with desiccant and close the bag while squeezing out the air. It can be stored for 1 month at 2 – 8°C).
 - 2) Dispense the negative control (R3), positive control (R4) and sample prepared with BSE refining kit into the microplate wells as shown below: When testing using several plates, place each control for each plate. When using 1 microplate in several tests, place each control for each measurement.
 - A1, B1, C1, D1: negative control (R3) 100µl
 - E1, F1: positive control (R4) 100µl
 - G1, H1: Sample 100µl
 - 3) Cover with sealing film and incubate at $37 \pm 1^\circ\text{C}$ for 75 ± 15 minutes using a heat block (desired), incubator, etc.
 - 4) Take off the sealing film and wash the plate with washing solution (18 – 22°C). When washing with an automatic microplate washer, use the overflow setting with 800µl per well with 3 washing cycles. To wash manually, repeat the process of removing the solution over wells and dispensing 350µl washing solution 3 – 6 times (adjust the number of washing cycles while observing the values). To remove the washing solution completely from the well after washing, tap out the solution on paper. Do not leave it in this condition for 5 minutes or

longer.

- 5) Dispense 100 μ l of the enzyme labeled antibody solution (R7) into each well.
- 6) Cover with sealing film and incubate for 60 ± 5 minutes at $2 - 8^{\circ}\text{C}$.
- 7) Take off the sealing film and wash the plate with washing solution ($18 - 22^{\circ}\text{C}$).
When washing with an automatic microplate washer, use the overflow setting with 800 μ l per well with 5 washing cycles. To wash manually, repeat the process of removing the solution over wells and dispensing 350 μ l washing solution 5 - 10 times (adjust the number of washing cycles while observing the values). To remove the washing solution completely from the well after washing, tap out the solution on paper. Do not leave it in this condition for 5 minutes or longer.
- 8) Dispense 100 μ l substrate coloring solution (R8 + R9) into the well and take measures to cover the plate with aluminum foil, etc. and incubate at room temperature ($18 - 22^{\circ}\text{C}$) for 30 minutes in light-tight dark room. Film must not be used in incubation.
- 9) Dispense 100 μ l of the reaction stopper solution (R10).
- 10) Within 30 minutes after adding the stopper solution, measure the OD at dominant wavelength 450 nm and subdominant wavelength¹ 620 nm using a microplate reader. Be sure to always shut off light until measurement is taken.

¹ The range of 600 - 700 nm for subdominant wavelength will not affect the judgment.

3. Judgment

Judgment shall use the cutoff value calculated as follows:

Cutoff value = (average absorbance for 4 negative controls + constant 0.210)

The constant is regularly reviewed. Use the value stated on the instruction manual for the kit.

Negative when OD value < -10% of the cutoff value

Re-testing required when OD value \geq -10% of the cutoff value

When re-testing is required by the above, use the 2 holes of the microplate and the stored sample in 1. (2) 4) (it is recommended that re-testing is conducted by a different inspector).

Furthermore, check the measurement system by checking that the absorbance values for negative and positive controls satisfy the following conditions:

- i) Absorbance values for all 4 holes with negative controls < 0.150
- ii) Absorbance values for both 2 holes with positive controls \geq 1.000

Judgment in re-testing shall be made as follows:

- (1) OD value for either of the 2 holes is equal to or larger than the cutoff value, or the OD value for either of the 2 holes is within -10% of the cutoff value, it is judged positive.
- (2) If OD values for both 2 holes are less than -10% of the cutoff value, it is judged negative.

(Appendix 1-2)

Procedure for Using “Dynabott Enfer BSE Test”

1. Kit components

(1) Components of Dynabott Enfer BSS Test

Reagent package (stored at 2 – 8°C)

Reagent	Volume	Storage condition	Reagent preparation method	Storage conditions after preparation/time limit for use
Reagent 3	20 mL x 1	2 – 30°C	N/A	N/A
Washing agent 1	100 g powder x 1 bottle	2 – 30°C	Add 50 g of washing agent 1 per 1L of purified water and dissolve.	6 months at 2 – 8°C
Goat serum	150µL x 1	2 – 8°C	See the section for anti-prion antibody.	N/A
Conjugate	Concentrated conjugate 100µL x 1	2 – 8°C	Dilute the conjugate to the specified scale with washing agent 2 solution for each lot.	To be used within 2 hours from preparation
Substrate A	10 mL x 1 bottle	2 – 8°C	Mix Substrates A and B in the same volumes.	Store in dark room and use on the day of preparation.
Substrate B	10 mL x 1 bottle	2 – 8°C		
Centrifuge plate	2 plates	2 – 30°C	N/A	N/A
Assay plate	1 plate	2 – 30°C	N/A	N/A
Positive control well	8 wells	2 – 8°C	N/A	N/A
Blank control	30 mL x 1	2 – 30°C	N/A	N/A

Antibody package (stored at -25 – -15°C)

Reagent	Volume	Storage condition	Reagent preparation method	Storage conditions after preparation/time limit for use
Reagent 2	3 mL x 1	-25 – -15°C	N/A	N/A
Anti-prion antibody (rabbit serum)	Concentrated antibody 50µL x 1 bottle	-25 – -15°C	Dilute the anti-prion antibody to 500-fold using washing agent 2 solution and dilute the goat serum to the specified scale for each lot.	Use on the same day of preparation.

Buffer/wash package (stored at 10 – 30°C)

Reagent	Volume	Storage condition	Reagent preparation method	Storage conditions after preparation/time limit for use
Reagent 1	1L x 1	10 – 30°C	N/A	N/A
Washing agent 2	10-fold concentrated solution 500 mL x 1 bottle	10 – 30°C	Add 100 mL washing agent per 900 mL purified water and mix.	2 weeks at 10 – 30°C 1 month at 2 – 8°C

(2) Ingredients and volumes

Component reagent	Ingredient	Content (in 100 mL)
Reagent 1	Methanol	16 mL
	Sodium lauryl sulfate (SDS)	15 g
Reagent 2	Proteinase K	0.2 g
Reagent 3	Guanidine hydrochloride salt	28.659 g
Washing agent 1	Sodium chloride	100 g ^{*1}
Washing agent 2	Laurocrogol	0.5 mL
Anti-prion antibody	Rabbit anti-prion serum	100 mL
Conjugate	Horseradish, peroxidase labeled anti-rabbit immunoglobulin (goat)	100 mL
Goat serum	Normal goat serum	100 mL
Positive control well	Synthetic prion peptides	2.4 ng ^{*2}
Substrate A	Substrate A (oxygenated water)	100 mL
Substrate B	Substrate B (3-aminophthalic hydrazide solution)	100 mL
Blank control	Methanol	16 mL
	Sodium lauryl sulfate	15 g
Assay plate	96-hole microplate	1 plate ^{*3}
Centrifuge plate	96-hole microplate	2 plates ^{*3}

^{*1}: Per 1 bottle^{*2}: Per 1 well^{*3}: Number of plates

2. Necessary tools and reagents

Materials contained in kit

- Reagents sufficient for testing 45 specimens are contained in the kit.

Materials not contained in kit

- High-grade deionized water, distilled water or reverse osmosis water shall be used (abbreviated as purified water hereafter).
- Stomacher Biomaster 80 (manufactured by Seward) homogenizer*
- Homogenizer bag (with filter) (manufactured by Interscience)

- Two units of Skatron SkanwasherR 300 (manufactured by Skatron) microplate washer*
 - iEMS incubator/shaker (manufactured by Thermo LabSystems)*
 - Luminoscan Ascent (manufactured by Thermo LabSystems) chemiluminescence measuring instrument*
 - Microplate centrifuge (2750G or higher)
 - Seal for microplate
 - Pipettes, etc.
 - Tools for specimen collection
 - Containers for diluting anti-prion antibody and conjugate
 - Glass or polypropylene containers for diluting other reagents
 - Negative control from tissue (see the section for preparation of tissue control.)
- * indicates specified devices required for this testing.

3. System parameter settings

The following provides the parameter information set for the recommended devices:
(It is not necessary for the user to set up.)

Washer

- This test requires 2 units of washers.
- For both Washing Protocols 1 and 2,
- Air pressure: 0.25 atm
- Volume/flow rate, adjustment offset >> ov: 1.00
- Aspirating position (normally 3.00 – 4.00 mm)
- Dispensing position: 0.00 mm

Washing Protocol 1*			Washing Protocol 2		
(Using Washing Agent Solution 1)			(Using Washing Agent Solution 2)		
Step:			Step:		
#1	Aspirate	6 sec	#1	Aspirate	4 sec
#2	Dispense	300μL	#2	Wash	3 sec
#3	Soak	5 sec	#3	Soak	5 sec
#4	Aspirate	4 sec	#4	Aspirate	2 sec
#5	Wash	5 sec	#5	Wash	3 sec
#6	Soak	5 sec	#6	Soak	5 sec
#7	Aspirate	3 sec	#7	Aspirate	2 sec
#8	Wash	2.5 sec	#8	Wash	3 sec
#9	Soak	5 sec	#9	Soak	5 sec
#10	Aspirate	2 sec	#10	Aspirate	2 sec
#11	Wash	2 sec	#11	Wash	2 sec
#12	Soak	5 sec	#12	Soak	5 sec
#13	Aspirate	5 sec	#13	Aspirate	4 sec
#14	End Wash		#14	End Wash	

* Operation of Washing Protocol 1 shall be conducted within a bio-safety cabinet.

Shaking incubator

- Shake value: 5 (1400 rpm), Temperature: 34°C

Chemiluminescence measuring instrument

- Plate acceleration: 10, Settle delay: 100, Filter: none, Measurement type: single,

Integration time: 300, Lag time: 30 sec, Measurement count: 1, Photomultiplier (PMT) voltage: default value, Plate type: 96 wells, Scale factor: $\cdot 8$

4. Preparation of reagents

Prepared reagents should be at room temperature at the time of use.

(1) Washing Agent Solution 1

Add 1 L purified water to 50 g of Washing Agent 1 (Enfer Wash 1) powder to prepare Washing Agent Solution 1. Shake until it dissolves (or set in rotary bottle shake for 10 minutes) and check that it is dissolved before using.

(Prepared Washing Agent Solution 1 can be stored for 6 months at 2 – 8°C.)

(2) Washing Agent Solution 2

Dilute the Washing Agent 2 (Enfer Wash 2) undiluted solution with purified water to 10-fold to prepare Washing Agent Solution 2.

(Prepared Washing Agent Solution 2 can be stored for 2 weeks at 10 – 30°C or 1 month at 2 – 8°C.)

(3) Anti-prion antibody + goat serum solution

Dilute the anti-prion antibody (Anti-PrP-1⁺ Ab (Rabbit)) and goat serum (Normal Goat Serum (Goat)) with Washing Agent Solution 2 and reverse and mix to prepare anti-prion antibody + goat serum solution.

Since the dilution scale differs by the lot, dilute according to the instructions on the bottle label.

(Use up the anti-prion antibody + goat serum solution within the day of preparation.)

(4) Conjugate solution

Dilute the conjugate (Enzyme-conjugate-2⁺ Ab (goat anti-rabbit)) with Washing Agent Solution 2 and reverse and mix to prepare the conjugate solution. Since the dilution scale differs by the lot, dilute according to the instructions on the bottle label.

(Store the prepared conjugate solution in a dark room and use up within 2 hours after preparation.)

(5) Substrate solution

Add Substrate A (Substrate Solution A) to Substrate B (Substrate Solution B) in equal volume.

Prepare the substrate solution at least 1 hour before use so that it is at room temperature when it is used.

(Store the prepared substrate solution in a dark room and use up within the day

of preparation.)

5. Preparation of specimen

- 1) Prepare the homogenate using 500 ± 40 mg of the collected bovine medulla (specimen).
- 2) Insert the specimen into a homogenizer bag (this bag is partitioned with a filter inside) in front of the filter and check that the specimen is pressed into the bottom of the bag. Mush the specimen with fingers to facilitate homogenizing.
- 3) Add 7.5 mL of Reagent 1 (Enfer Buffer 1 (Bovine)) into the deeper side of the filter in the homogenizer bag. Although there is no stipulation regarding the period until homogenizing after Reagent 1 is added, consideration should be given so that it is put through the immunoassay step smoothly.
- 4) Set the speed of Stomacher homogenizer to "high" to homogenize the specimen for 2 minutes. Since emulsion is prepared using a homogenizer bag with a filter, unnecessary parts such as membrane will be removed.

Note: Put the homogenized specimen through the immunoassay step immediately after homogenizing.

Store the residue from the emulsion used in testing in the homogenizer bag at room temperature until the first test result is obtained. Do not refrigerate since it will cause crystallization.

6. Procedure for immunoassay

- 1) Leave Positions A1 and A2 on centrifuge plates as positive control wells (Peptide Indicator Wells).
Dispense blank control (Blank Control Reagent (Bovine)) in 4 wells from Position B1 and 2 wells for each specimen at the volume of 180 μ L each.
- 2) Cover the centrifuge plate with a seal.
- 3) Centrifuge the centrifuge plate for 5 minutes at 2750 G.
- 4) Dispense 20 μ L of Reagent 2 (Enfer Buffer 2) into the bottom of each well of the assay plate (Enfer Test Plate) to be used in measurement.
- 5) Remove the seal from the centrifuge plate for which centrifuge has been completed to collect the 100 μ L supernatant for each specimen and blank control and transfer to the assay plate with Reagent 2.
- 6) Cover the assay plate with a seal.
- 7) Shake the assay plate for 60 minutes at 34°C.
- 8) Remove the seal and wash the assay plate using Washing Agent Solution 1 and

- (Washing Protocol 1).
- 9) Turn over the assay plate on soft paper and tap well to remove the remaining liquid.
 - 10) Add 150 μ L of Reagent 3 (Enfer Buffer 3) on each well.
 - 11) Cover the assay plate with a seal.
 - 12) Shake the assay plate for 15 minutes at 34°C.
 - 13) Remove the seal and wash the assay plate using Washing Agent Solution 2 and (Washing Protocol 2).
 - 14) Turn over the assay plate on soft paper and tap well to remove the remaining liquid.
 - 15) Remove Wells A1 and A2 from the assay plate to replace with the positive control well.
 - 16) Dispense 150 μ L of the prepared anti-prion antibody + goat serum solution into each well.
 - 17) Cover the assay plate with a seal.
 - 18) Shake the assay plate for 40 minutes at 34°C.
 - 19) Remove the seal and wash the assay plate using Washing Agent Solution 2 and (Washing Protocol 2).
 - 20) Turn over the assay plate on soft paper and tap well to remove the remaining liquid.
 - 21) Dispense 150 μ L of the prepared conjugate solution into each well on the assay plate.
 - 22) Cover the assay plate with a seal.
 - 23) Shake the assay plate for 30 minutes at 34°C.
 - 24) Remove the seal and wash the assay plate using Washing Agent Solution 2 and (Washing Protocol 2).
 - 25) Turn over the assay plate on soft paper and tap well to remove the remaining liquid.
 - 26) Dispense 150 μ L of the prepared substrate solution into each well on the assay plate.
 - 27) Cover the assay plate with a seal.
 - 28) Shake the assay plate for 10 minutes at 34°C.
 - 29) Remove the seal and read the luminescence strength using the chemiluminescence-measuring instrument.

Example of assay plate (Enfer Test Plate) arrangement

	1	2	3	4	5	6	7	8	9	10	11	12
A	P	P	S6	S6	S14	S14	S22	S22	S30	S30	S38	S38
B	B	B	S7	S7	S15	S15	S23	S23	S31	S31	S39	S39
C	B	B	S8	S8	S16	S16	S24	S24	S32	S32	S40	S40
D	S1	S1	S9	S9	S17	S17	S25	S25	S33	S33	S41	S41
E	S2	S2	S10	S10	S18	S18	S26	S26	S34	S34	S42	S42
F	S3	S3	S11	S11	S19	S19	S27	S27	S35	S35	S43	S43
G	S4	S4	S12	S12	S20	S20	S28	S28	S36	S36	S44	S44
H	S5	S5	S13	S13	S21	S21	S29	S29	S37	S37	S45	S45

P: Positive control wells (Peptide Indicator Wells)

B: Blank control (Blank Control Reagent (Bovine))

S1 – S45: Samples

Flow of Measurement Procedure

Step	Process	Period/ temperature	Device/equipment used	Preparation for the next process	Precautions
Collection of specimen/preparation process					
Collection of specimen/weighting	Weighing the specimen: 500 \pm 40 mg		Balance		
Homogenizing	Add 7.5 mL Reagent 1 per 500 \pm 40 mg tissue	2 min (Speed "High")	Stomacher 80 homogenizer		Insert the tissue sample into the bottom of filter bag. Let it stand at room temperature until bubbles disappear from the sample when finished.
Transferring the homogenate	Transfer the homogenate to centrifuge plate: 180 μ L		Pipette	Transfer 180 μ L of the blank control to plate as well.	Cover the plate with seal
Centrifuge	Centrifuge: 2,750 G	5 min Room temperature	Centrifuge	Check that the incubator temperature is 34°C.	Ensure balance before centrifuging. Centrifuge at 2 - 8 °C is strictly prohibited.
Addition of Reagent 2 (PK)	Add into assay plate: 20 μ L.		(8-gang) pipette		Add to the corner of well bottom (check addition of Reagent 2 visually at the end).
Dispensing the specimen into plate	Add centrifuge supernatant to plate: 100 μ L.		(8-gang) pipette		Collect supernatant with caution on precipitate.
Incubation 1	Incubation	60 min at 34°C	LabSystem iEMS incubator		
Washing 1	Wash with Washing Agent 1 under Protocol 1.		Skanwasher 300 washer		After washing, turn over and tap several times over paper towel to

					remove remaining liquid.
Addition of Reagent 3	Add 150µL of Reagent 3.		(8-gang) pipette		
Incubation 2	Incubation	15 min at 34°C	LabSystem iEMS incubator	Preparation of the first antibody solution (dilute anti-prion antibody and goat serum with prepared Washing Agent Solution 2.) Preparation of conjugate (second antibody) solution (dilute the conjugate with prepared Washing Agent Solution 2.) Also, prepare the substrate solution.	
Washing 2	Wash with Washing Agent solution 2 under Protocol 2.		Skanswasher 300 washer	After washing, break off Wells A1 and A2 and set the positive control wells.	After washing, turn over and tap several times over paper towel to remove remaining liquid.
ELISA process					
First antibody	Addition of the first antibody: 150µL		(8-gang) pipette		Check that the positive control is placed at Positions A1 and A2 and then add the first antibody.
Incubation 3	Incubation	40 min at 34°C	LabSystem iEMS incubator		
Washing 3	Wash with Washing Agent solution 2 under Protocol 2.		Skanswasher 300 washer		After washing, turn over and tap several times over paper towel to

					remove remaining liquid.
Conjugate	Addition of conjugate: 150 μ L		(8-gang) pipette		
Incubation 4	Incubation	30 min at 34°C	LabSystem iEMS incubator		
Washing 4	Wash with Washing Agent solution 2 under Protocol 2.		Skawasher 300 washer		After washing, turn over and tap several times over paper towel to remove remaining liquid.
Substrate	Addition of substrate: 150 μ L		(8-gang) pipette		
Incubation 5	Incubation	10 min at 34°C	LabSystem iEMS incubator		
Measurement	Measurement of chemiluminescence		Luminometer		

7. Judgment method

(1) Verification of test performance

It is necessary that the control results be verified before judging the specimen results. Average luminescence strength values for the blank control and positive control wells should be obtained. If the following standards are not satisfied, assay results are invalid. Conduct re-testing from the process of bovine medulla (specimen) collection described in "5. Preparation of specimen." In this case, measurement results are judged using the 2 wells as well.

1) Blank control

The median value for measurement using the 4 blank control wells must be less than 4.0LU. The median value is calculated by averaging the 2 measurement values that are not the largest or smallest for the 4 wells.

2) Positive control well

After subtracting the median value for the blank control, check that the average value of positive control wells stays inside the control range for the positive control wells from the lot used (stated on the label for the positive control wells).

The individual measurement values for positive control wells must not exceed $\pm 30\%$ from the average value of the positive control wells.

- (2) The cutoff value for this kit is 5.5LU. In addition, measurement values for all samples shall be used for judgment after subtracting the median value for blank controls.

When the measurement values for the 2 wells are both 5.5LU or smaller, it is judged as negative according to this kit. On the other hand, if a value exceeding 5.5LU is obtained in at least 1 of the 2 wells, the specimen needs to be re-tested with a test using 2 wells from the process of bovine medulla (specimen) collection described in "5. Preparation of specimen."

If at least one of the measurement values using 2 wells exceeds 5.5LU as the result of re-testing, it is considered positive according to this kit, and a confirmatory test is required since it is suspected to be positive. If both of the measurement values using 2 wells in re-testing are 5.5LU or smaller, it is considered negative according to this kit.

Specimen measurement results (n = 2)

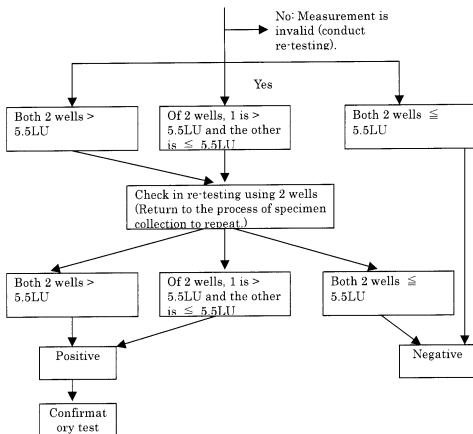
Blank control

- The median value for quadruple blank control measurement must be less than 4.0LU. The median value is calculated by averaging the 2 measurement values that are not the largest or smallest for the 4 wells.

Positive control wells

(Applied only when positive control wells accessory to this kit are used.)

- After subtracting the median value for the blank control, check that the average value of positive control wells stays inside the control range for the positive control wells from the lot used.
- The individual measurement values for positive control wells must not exceed $\pm 30\%$ from the average value of the positive control wells.



8. Dividing the kit

If the number of specimens is small, it is possible to divide the kit into 4 different tests. The minimum number of specimens in this case is 1.

9. Treatment in relation to specimen judged as positive in this test method

If re-testing is required as the result of judgment, move the emulsion that is supposed to be stored in the homogenizer bag at room temperature until judgment is made to a 15 ml plastic centrifuge tube for cultivation and store by freezing.

If it is positive as the result of re-testing, move the emulsion used in re-testing to a 15 ml plastic centrifuge tube and freeze for use in the confirmatory test along with the stored frozen emulsion from the first test.

If transport is necessary in implementing the confirmatory test, fix the lid of the 15 ml plastic centrifuge tubes for cultivation that contain the emulsion with Parafilm and wrap the entire tube with tissue, etc. as absorbing material in case the plastic centrifuge tubes are broken or the cap is removed and as cushioning material for shock. Then include in a biohazard can, etc. to ship as a specimen to be sent for western blot testing.

If both wells are negative as the result of re-testing, the stored frozen emulsion from the initial test should be discarded.

Procedure for Confirmatory test on Bovine Spongiform Encephalopathy (BSE) in Prefectures, etc.

1. Institute for test implementation

- (1) Inspection and Safety Division specifies the testing institutes of prefectures, etc. that satisfy the requirements in (2) as “BSE confirmatory test institutes.”
- (2) Requirements for specification
 - a) Any person who has undergone the technical courses in relation to the BSE confirmatory test provided by the Inspection and Safety Division or any person who is approved by the Inspection and Safety Division as having equivalent or better competence and belongs to the institute.
 - b) Necessary devices, etc. indicated in the procedure for this confirmatory test are prepared.
 - c) The test method in 2 is observed.
 - d) The test technique will be checked as in external precision control notified separately.

2. Implementation of confirmatory test

- (1) For western blot method, the test should be implemented according to Appendix 2-1 “Procedure for immunobiochemical test (western blot method)” and the confirmatory test to be conducted in the prefecture, etc. should be implemented only once.

Furthermore, if satisfactory test results cannot be obtained, the specimen should be set to the National Institute of Infectious Diseases, etc. for the confirmatory test.

When sending a specimen, the stored specimen frozen as material for the immunobiochemical test (ELISA method and western blot method) and any remaining sample from the immunobiochemical test in a frozen condition should be sent according to the method of sending a specimen for the confirmatory test.

- (2) For the immunohistochemical test and the histological test, the test should be conducted by the prefecture, etc. according to Appendix 2-2 “Procedure for immunohistochemical test,” and the specimen should be sent to the National Institute of Infectious Diseases, etc. for the confirmatory test.

In addition, remains from the section to be cut out A (A*) – C (C*) illustrated in Figure 1 of Appendix 2-2 Procedure for immunohistochemical test should be included as all sections to be sent in a 50 ml container filled with formalin buffer at room temperature.

(Appendix 2-1)

Procedure for Immunobiochemical Test (Western Blot Method)

1. Instruments, etc.

- Electrophoresis bath: 1 set of XCell SureLock Mini-Cell (Invitrogen EI0001)
- Blotting bath: Mini Trans-Blot Cell (Bio-Rad, 170-3930)
- Power supply: Power Pack 200 (Bio-Rad, 165-5052)
PowerEase 500 power Supply (Invitrogen EI8600)
- Membrane roller: Membrane roller (Advantech, EBA-200)
- Ultrasonic generator: One with output 750 W or larger or with equivalent output by booster effect (Ex.: Digital sonifier S450D by Bramson)
- Multi-Beads Shocker: Original product by Yasui Kikai Corporation
- Constant-temperature bath (water bath): A water bath that can be used at 37°C (cooling function desired)
- Balance: With minimum weighing unit of 10 mg or smaller
- High-speed refrigerated microcentrifuge: One that is operable at 15,000 rpm or higher

2. Reagents, etc.

- | | | |
|---|--------------------------------|--|
| - Collagenase (for cell dispersion grade) | Wako | 100 mg, No.038-10531 |
| - Pefablock | Roche | 500 mg, No.1585916 |
| - Proteinase K, PCR grade | Roche | 5 ml, No.1964372 |
| - Dnase I | Roche | 100 mg, No.104159 |
| - N-Lauroylsarcosine (Sarkosyl) | Sigma | 100 g, No.L-5125 |
| - Zwittergent 3-14 | Calbiochem | 5 g, No.693017 |
| - Sodium dodecyl sulfate (SDS) | Sigma | 500 g, No.L-4509 |
| - 2-mercaptoethanol | Sigma | 100 ml, M-6250 |
| - Urea (special reagent class) | Wako | 500 g, 217-00615 |
| - 2-Butanol | Wako | 500 ml, 020-11215 |
| - Tween 20 | Wako | 500 ml, 167-11515 |
| - Skim milk | COOP, Meiji, Yukijirushi, etc. | |
| - Fetal bovine serum (FBS) | <u>Any manufacturer</u> | |
| - Immobilon-PVDF | Millipore | No.IPVH00010 |
| - Filter paper | | Bio-Rad 7.5 x 10 cm, No.170-3932
ADVANTEC 60 x 60 cm, No.514A |

· X-ray film	Fuji Film	6-cut, No.03D051
· ECL western blotting detection reagent	Amersham Pharmacia	No.RPN2209
· Anti-rabbit IgG HRP conjugated	Amersham Pharmacia	1ml, NA9340
· Anti-mouse IgG HRP conjugated	Amersham Pharmacia	1ml, NA9310
· 2ml tube with O-ring	Asyst	No.72.693S

(This is not a tube for Multi-Beads Shocker.)

3. Preparation of reagents

- TN buffer: 100 mM NaCl, 50 mM Tris-HCl (pH 7.5)
- Detergent buffer: 4% Zwittergent 3-14, 1% Sarkosyl,
100 mM NaCl, 50 mM Tris-HCl (pH 7.5)
- Butanol-Methanol mixture: 2-Butanol:Methanol = 5:1(v/v)
- Proteinase K: 1 mg/ml in 50 mM Tris-HCl (pH 8.0), 1 mM CaCl₂,
Stored at -20°C by dispensing.
- Pefablock: 0.1M in DDW, stored at -20°C by dispensing.
- Collagenase: 20 mg/ml in DDW, stored at -20°C by dispensing.
- DNase 1: At 10 mg/ml concentration, 50% glycerol, 10 mM Tris-HCl (pH 7.5)
Stored at -20°C by dissolving in 10 mM MgCl₂.
- Sample buffer (x 1): 62.5 mM Tris-HCl (pH 6.8), 5% glycerol, 3 mM EDTA, 5% SDS,
4M Urea, 4% β-mercapthoethanol, 0.04% bromo phenol blue,
A small amount in use can be stored at room temperature.
A temperature of 4°C is recommended for long-term storage (Although
Urea and SDS may precipitate, they can be dissolved by heating to about
50°C before use).

1 M Tris-HCl (pH 6.8)	1.25 ml
Glycerol	1 ml
0.5M EDTA (pH 8.0)	120μl
β-Mercapthoethanol	800μl
1% bromo phenol blue	800μl
SDS	1 g
<u>Urea</u>	<u>4.8 g</u>

Up to 20 ml

4. Preparation of brainhomogenate

- When using the Multi-Beads Shocker (by Yasui [Kikaji](#)),

- 1) Insert the metal cone (No.MC-01212PP) in the special tube with 2 ml O-ring.
 - 2) Put 200 mg of the brain tissue into the tube.
 - 3) Add 800 μ l of TN buffer.
 - 4) Shake for 30 seconds with the Multi-Beads Shocker at 2000 rpm.
 - 5) This is used as 20% (W/W) brain homogenate and it is stored in a tube with O-ring.
- b) When using an sonicator,
- 1) Mince 200 mg brain tissue on Parafilm and transfer to a 2 ml tube.
 - 2) Add 800 μ l of TN buffer.
 - 3) Processwith ultrasonic using a cup horn-type sonicatoruntil the tissue become a uniform homogenate.
 - 4) This is used as 20% (W/W) brain homogenate and it is stored in a tube with O-ring.
- c) When using Enfer method Stomacher homogenizer,
- 1) Insert 500 \pm 40mg of brain tissue into the homogenizer bag.
 - 2) Add 7.5 ml of Enfer kit Reagent 1 (Enfer Buffer 1 (Bovine)).
 - 3) Process this with Stomacher homogenizer for 2 minutes at speed High.
 - 4) This is used as 6.25% (W/W) brain homogenate and it is stored with division into small volume of 1 ml.

5. Preparation of specimen

- 1) Add 250 μ l of Detergent buffer to the 250 μ L of 20% (W/W) brain homogenate in a 2 ml tube with O-ring and provide vortex (combine with ultrasonic processing if necessary) (Note 1).
- 2) Add μ l of 20 mg/ml collagenase and vortex.
- 3) Digest for 30 minutes at 37°C (be sure to conduct this in water bath).
- 4) Add 20 μ l of 1 mg/ml PK and vortex.
- 5) Digest for 30 minutes at 37°C (be sure to conduct this in water bath and provide vortex once of twice during digestion).
- 6) Add 10 μ l of 0.1M Pefablock and vortex.
- 7) Add 2 μ l of 10mg/ml DNase and vortex then left to stand for 5 minutes at room temperature.
- 8) Add 250 μ l Butanol-Methanol mixture then vortex.
- 9) Centrifuge at 15000rpm for 10 minutes at 20°C.
- 10) Remove the supernatant and air dry the precipitate briefly(Note 2).
- 11) Add 100 μ l of 1x Sample buffer and boil for 5 minutes at 100°C.

If precipitate does not dissolve easily, process with sonication.

- a) Preparation of 20% brain homogenate prepared with BSE purification kit (Bio-Rad)
- 1) Add 250 μ l of detergent buffer to 250 μ l of 20% brain homogenate and vortex and ultrasonic processing (Note 1).
 - 2) Add 12.5 μ l of 20 mg/ml collagenase then vortex.
 - 3) Digest for 30 minutes at 37°C.
 - 4) Add 20 μ l of 1 mg/ml PK then vortex.
 - 5) Digest for 30 minutes at 37°C.
 - 6) Add 10 μ l of Pefablock then vortex.
 - 7) Add 250 μ l of Butanol-Methanol mixture.
 - 8) Provide vortex.
 - 9) Centrifuge at 15,000rpm for 10 minutes at 20°C.
 - 10) Remove the supernatant and air dry the precipitate (Note 2).
 - 11) Add 100 μ l 1x Sample buffer and boil for 5 minutes at 100°C.

If precipitate does not dissolve easily, process with sonication.

- (Note 1) Digestion of non-specific proteins by proteinase K is facilitated and western blot result will be more clear if 25 μ l (5%) 2-butanol is added to the reaction mixture.
- (Note 2) Since supernatant contains butanol, it must be treated as an organic solvent. Add 10 N NaOH of 1/10 volume for prion deactivation and let it stand for 2 hours or longer and then neutralize.

- b) Preparation of samples from the 6.25% brain homogenate prepared by Stomacher homogenizer (Enfer method)
- 1) Centrifuge 1 ml brain homogenate for 10 minutes at 20°C and 15,000 rpm and transfer 800 μ l of the supernatant (equivalent to 50 mg tissue) to another 2 ml tube.
 - 2) Add 20 μ l of 20 mg/ml collagenase then vortex.
 - 3) Digest for 30 minutes at 37°C.
 - 4) Add 20 μ l of 19.2 mg/ml PK for vortex.
 - 5) Digest for 30 minutes at 37°C.
 - 6) Add 16 μ l of 0.1 M Pefablock for vortex.
 - 7) Add 3.4 μ l of 10 mg/ml DNase then vortex and let it stand for 5 minutes at room temperature.
 - 8) Add 400 μ l 2-Butanol solution then vortex.
 - 9) Centrifuge at 15,000rpm for 10 minutes at 20°C.

- 10) Remove the supernatant and turn the sample tube over onto a paper towel and let it stand for about 5 minutes to dry the precipitate.
- 11) Add 100μl 1x Sample buffer and boil for 5 minutes at 100°C. If the precipitate does not dissolve easily, process with sonication.

※ Decontamination of equipment, etc. used in sample preparation

- Scissors, forceps, chips, tubes, etc. in a pressure-resistant and heat-resistant container for autoclave for 30 minutes at 135°C. In this case, add about 150ml water in the container and do not close the lid.
- Decontaminate inflammables by autoclaving as well.

6. SDS-PAGE

- Use the precast gel by Invitrogen (former Novex).
- Gel: NuPAGE 12% Bis-Tris Gel, 1.0 mm, 12 well (Invitrogen, No.NP0342)
- Use the gel loading chip (Funakoshi SRPT-1381) to load 20μl (equivalent to 10 mg tissue) or 5μl (equivalent to 2.5 mg tissue).
- Buffer: NuPAGE MOPS SDS Running buffer (Invitrogen No.NP0001). Add 500μl Antioxidant (Invitrogen No.NP0005) to the cathode side buffer.
- Conduct electrophoresis at constant voltage of 200 V.

<Positive control for sensitivity measurement>

Use 10-fold dilution of positive control (MoPrP^{Sc} lot 011209, equivalent to 10 mg/ml tissue) with the sample buffer as the original solution (4⁰, equivalent to 100 ug/10μl tissue). Heat the original solution (4⁰) at 100°C for 2 minutes once when it is prepared. Store the original solution at -20°C by dispensing to about 50μl/tube. Then prepare 4 serial dilution of 4⁻¹ (25 ug/10μl), 4⁻² (6.25 ug/10μl), 4⁻³ (1.6 ug/10μl), and 4⁻⁴ (0.4 ug/10μl) and store them at -20°C by dispensing. Dissolve the samples at 50°C before use. Load the 4⁻¹ to 4⁻⁴ positive controls at 10μl/lane (4⁻² to 4⁻⁴ is also acceptable depending on the conditions of lane use). Since these positive control dilution lanes are required for WB sensitivity evaluation, conduct electrophoresis on the same gel as the sample. If PrP^{Sc} is detected up to 4⁻³, the result can be evaluated.

Positive control is distributed by Prof. Horiuchi of Hokkaido Univ. etc.

7. Western Blot (WB)

- Blotting cell: Bio-Rad, Mini Trans-Blot Cell (170-3930)
- Transfer buffer

NuPAGE transfer buffer (Invitrogen No.NP0006)	50 ml	
Antioxidant (Invitrogen No.NP0005)	1 ml	
Methanol	200 ml	final 20%
<u>20% SDS</u>	<u>0.5 ml</u>	final 0.01%
Up to 1 L		

- Cut a PVDF membrane (Immobilon-PVDF) to 7.5 x 9 cm and soak in methanol for 1 minute to activate it. Then wash with DDW and soak it in the transfer buffer.
- Soak the gel for which electrophoresis has been completed in the transfer buffer.
- Place the PVDF membrane on 2 pieces of filter papers soaked in transfer buffer (1 piece if Bio-Rad filter paper is used) and place the gel on it. Use caution not to let air bubbles form between the gel and PVDF membrane. Then place 2 pieces of filter paper soaked in transfer buffer on the gel.
- Insert the above filter paper-PVDF-gel-filter paper sandwich between blotting pads and set it on the blotting system. Since protein moves from cathode to anode, set it so that the PVDF membrane is at the anode side of the gel.
- Conduct blotting under the conditions of a) – c). a) or b) is recommended. If it must be conducted in haste, c) is acceptable but has a tendency to have high background.
 - a) 6 – 15 hours at constant voltage of 30 V
 - b) 2 hours at constant voltage of 60 V
 - c) 1 hour at constant voltage of 80 V

8. Immunostaining

It is desired that 44B1 is used as the primary method and B103 as the secondary.

- [1] When using 44B1 monoclonal antibody (Note 3) (Note 4),
 - 1) Blocking: 5% skim milk, 5% FBS in PBST (0.1% Tween 20). Be sure to heat and dissolve skim milk (at about 80°C). After cooling, add FBS was added to the final concentration of 5% (Since 44B1 has a tendency to have high background for PVDF membrane, FBS is added to provide a blocking effect.)
 - 2) Incubate for 1 hour on membrane roller (Advantec, No.EBA-200).
 - 3) Primary antibody: Dilute with 1% skim milk, 1% FBS in PBST. Guideline for concentration of the antibody is 0.1 – 0.2 µg/ml.
 - 4) 1 hour on membrane roller.
 - 5) Wash with PBST for 20 minutes. Replace with PBST 5 times.
 - 6) Secondary antibody (Amersham NA9310): Dilute to 1:2,500 with 1% skim milk

and 1% FBS in PBST.

- 7) Incubate for 45 minutes on membrane roller.
- 8) Wash with PBST for 20 minutes. Replace with PBST 5 times.
- 9) Provide luminescence with ECL western blotting detection reagent.
- 10) Expose to X-ray film for 2 minutes and develop the film.
- 11) Expose to another X-ray film while developing.
- 12) Develop after 30 minutes (prepare X-ray films with 2-minute and 30-minute exposures) (Note 5).
- 13) Then expose overnight if necessary.
 - Developing solution: Hi Rendol
 - Stop solution: 3% acetic acid
 - Fixing solution: Super Fuji Fix

[2] When using B103 affinity-purified polyclonal antibody (Note 6),

(Differs from [1] 44B1 in 1), 3) and 6).)

- 1) Blocking: 5% skim milk in PBST (0.1% Tween 20). Be sure to heat and dissolve Skim milk (at about 80°C).
- 2) Incubate for 1 hour on membrane roller (Advantec, No.EBA-200).
- 3) Primary antibody: Dilute with 1% skim milk in PBST. Guideline for concentration of B103 is 1 µg/ml.
- 4) Incubate for 1 hour on membrane roller.
- 5) Wash with PBST for 20 minutes. Replace with PBST 5 times.
- 6) Secondary antibody (Amersham NA9340): Dilute to 1:2,500 with 1% skim milk.
- 7) Incubate for 45 minutes on membrane roller.
- 8) Wash with PBST for 20 minutes. Replace with PBST 5 times.
- 9) Provide luminescence with ECL western blotting detection reagent.
- 10) Expose to X-ray film for 2 minutes and develop the film.
- 11) Expose to another X-ray film while developing.
- 12) Develop after 30 minutes (prepare X-ray films with 2-minute and 30-minute exposures) (Note 5).
- 13) Then expose overnight if necessary.
 - Developing solution: Hi Rendol
 - Stop solution: 3% acetic acid
 - Fixing solution: Super Fuji Fix

(Note 3) 44B1 antibody is distributed by Prof. Horiuchi of Hokkaido University, etc.
The current lot is 02011, 6.5 mg/ml.

(Note 4) If the background for PVDF membrane is high in the above procedure and specified sensitivity (see p.18) cannot be obtained, it can be improved by the following method:

- ① Change the blocking solution and antibody reaction solution to 5% skim milk in 50 mM Tris-HCl (0.1% Tween 20) and 1% skim milk in 50 mM Tris-HCl (0.1% Tween 20), respectively.
- ② Wash the membrane after antibody reaction with 0.1% Tween 20 in PBS (50ml) for 5 minutes x 6 times.
- ③ Use Invitrogen transfer system (Excel II blot module E19051), transfer buffer (NuPAGE transfer buffer: NP0006, NP006-1) and PVDF membrane (LC2005) to transfer for 1 hour at 20 V.

(Note 5) 30 minutes is only a guideline, and it shall be modified flexibly depending on the results of 2-minute exposure.

(Note 6) B103 antibody is available from Fujirebio, Inc. The current lot is SB21103, 1 mg/ml.

Reference: Example of test flow

[Ex.1] Day 1: Preparation of specimen (2 hours)

Day 2: PAGE (1.5 hours) → WB (2 hours) → Staining (4 hours)

[Ex.2] Day 1: Preparation of specimen (2 hours) → PAGE (1.5 hours) → WB (up to 2 hours)

Day 2: Staining (4 hours)

[Ex.3] Day 1: Preparation of specimen (2 hours) → PAGE (1.5 hours) → WB (2 hours) → Staining (4 hours)

9. Precision control

- 1) Internal precision control should be implemented at the frequency of every month using the positive control distributed by Prof. Horiuchi of Hokkaido University, etc. and the samples confirmed as negative in the screening test.
- 2) External precision control should be implemented as notified separately.

3. Firm diagnosis

- 1) The institutes implementing the confirmatory tests should send the confirmatory test data to the Inspection and Safety Division by electronic media. Furthermore, when conducting confirmatory tests at the prefecture, etc., the corresponding prefecture, etc. should send the confirmatory test data to the Inspection and Safety

Division by electronic media.

- 2) The Inspection and Safety Division should send the confirmatory test data to the members of the "Expert Committie for BSE Diagnosis" for firm diagnosis.
- 3) If necessary, obtain a specialist's diagnosis of the histopathological sample (staining sample) using microscopy.
- 4) The results of firm diagnosis should be informed to the prefecture, etc. implementing the confirmatory test from the Inspection and Safety Division.

Procedure for Histology and Immunohistochemistry

1. Preparation of paraffin block

<Preparation>

Disposable bench sheet (laboratory sheet), cutting board, blade for cutting, tweezers, stainless tray, numbered plastic cassettes in the required quantity, can be used for blade disposal, Kimtowel, 1N NaOH, container for formic acid treatment

<Procedure>

- 1) Cut out the formalin-fixed tissue in the safety cabinet whilst wearing the specified clothing (conforms to the clothing for the screening test).
- 2) Wipe the outer surface of the specimen container with 1N NaOH and then wipe with a cloth soaked in water.
- 3) Cut out the formalin-fixed tissue.

Spread a laboratory sheet and place a plastic cutting board, then cut out the tissue on the plastic cutting board with a disposable blade. The thickness for the cut tissue in the obex should be 3 mm or smaller. Cut out the obex first and more 2 pieces from the above to make a total of 3 pieces and place them into a plastic cassette.

For sufficient fixation, shake in 15 – 20% formalin solution for at least 1 hour at 60°C (for rather unfixed material, 2 – 3 hours for fixation are desired). When processing for overnight, fix at 37°C for 1 hour and 30 minutes before the embedding process.

- 4) Treat with formic acid for 1 hour at room temperature.

Drop the fixed brain tissue in the plastic cassette directly in 98% formic acid solution and shake for 1 hour in a shaker at room temperature. Wash with running water for 30 minutes (to lower infectiousness).

- 5) Process with sealed automatic embedding system in a 4.5-hour protocol or by manual rotation.
- 6) Use the system and mold for paraffin embedding which are limited for use of BSE samples .

<Post-processing>

- 1) Discard formalin in a special waste liquid tank. Incinerate later.
- 2) Tweezers and blades used in cutting should be placed on the stainless tray to soak in 1N NaOH for 2 hours at room temperature (or place in special can for autoclaving later). Then wash with water. Discard the blades.
- 3) Cover the cutting board with Kimtowel soaked in 1N NaOH and leave it for 2 hours and then wash with water.
- 4) Return the remaining brain tissues to the fixing bottle for storage. When they are no longer necessary, put them on autoclave processing (see below) and then discard.

2. Section preparation

<Preparation>

Bench sheet, microtome, bucket of water, paraffin stretcher, humidifier, can for replaced blade disposal, slide glass (silane-coated slide), 1N NaOH, Kimtowel

<Procedure>

- 1) Wear gloves, shielded mask, and gown. If necessary, use the anti-cut gloves.
- 2) Spread the bench sheet and place microtome. Prepare a paraffin stretcher and a bucket of water for BSE samples and mount the section on the silane-coated slide.
- 3) Aspirate the remaining pieces during section preparation with a special vacuum equipped with HEPA filter. Incinerate or autoclave later.
- 4) Autoclave the knife holder for 60 minutes at 135°C and then wash with water and dry.
- 5) Soak the blades in 1N NaOH at room temperature (or place in a special can and autoclave later).
- 6) Dry the sections completely at 45°C.

<Post-processing>

Aspirate all remaining paraffin pieces using the previously described vacuum.

3. HE staining (Prepare a special series of Copring jar (container) for paraffin removal and staining)

- 1) Deparaffinization in xylen, through graded ethanol, and immerse in DDW.
- 2) Harris' hematoxylin staining for 2 minutes at room temperature.

- 3) Place the sections in warm water for 10 minutes to get clear color.
- 4) Eosin staining for 3 minutes at room temperature.
- 5) Clarification, dehydration and clearing in xylene.
- 6) Mount with a cover glass.

4. Immunohistochemistry

<Reagents>

Envision +kit (DAKO, for mouse and rabbit antibodies),
 Simple stain DAB solution (Histofine), 3% hydrogen peroxide in water
 Primary antibody to prion, Hematoxyline, PBS

Preparation of PB (0.1M PB for immunity)

$\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$	28.7 g
$\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$	3.3 g
D.W. (distilled water)	1.0 L

Preparation of PBS (0.01M PBS)

PB	100 ml
D.W. (distilled water)	900 ml
NaCl	8.5 g

<Procedure>

- 1) Deparaffinization in xylene, through graded ethanol, and immerse in DDW.
- 2) Place the sections in distilled water in stainless vat, and autoclave with the specified autoclave for 20-minute at 121°C. Transfer the sections in PBS when temperature decreases.
- 3) Blocking endogenous peroxidase activity by 3% hydrogen peroxide in DDW for 5 minutes at room temperature.
- 4) Blocking with 10% normal goat serum in PBS for 5 minutes at room temperature (omission possible).
- 5) Apply primary antibody (described later) and react for 30 – 40 minutes.
- 6) Wash with PBS.
- 7) After reaction in Envision+ solution for 30 minutes at room temperature, wash with PBS.
- 8) Conduct DAB coloring reaction.

- 9) After washing with tap water and nuclear staining with Mayer's hematoxylin for 30 seconds at room temperature.
- 10) Clearing in warm water, dehydration, clearing in xylene and mount.

<Post-processing>

Xylene for paraffin removal, ethanol and distilled water shall be discarded in separate special containers to be incinerated.

Process the special staining vat and basket at 135°C for 1 hour and then wash with water.

Discard the staining solutions such as hematoxylin and eosin.

5. Precision control

- 1) Implement internal precision control at the frequency of every month by using the positive control distributed from the National Institute of Infectious Diseases, etc. and samples confirmed as negative in screening test.
- 2) Implement the external precision control as notified separately.

1. Illustration of paraffin block preparation (1)
: From embedding (manual)

After cutting, re-fixing by soaking and shaking in 20% formalin for 60 minutes at 60°C

Soaking for 60 minutes in 98% formic acid

Washing in running water for 30 minutes

Removal of excess water with filter paper

80% Ethanol 15 minutes

90% Ethanol 15 minutes

100% Ethanol 15 minutes

100% Ethanol: Acetone

(1:1) 15 minutes

Acetone 15 minutes

Xylene 15 minutes

Xylene 15 minutes

Xylene 15 minutes

Paraffin 15 minutes

Paraffin 15 minutes

Paraffin 15 minutes

Embedding

Required period: 2 hours and 45 minutes

(However, cells tends to be shrinkage.)

1. Illustration of paraffin block preparation (2)
- : Processing with automatic embedding system

System used: Sakura automatic sealed fixing embedding system

	Set period
80% Ethanol	10 minutes
90% Ethanol	10 minutes
95% Ethanol	10 minutes
99% Ethanol	20 minutes
100% Ethanol I	20 minutes
100% Ethanol II	30 minutes
100% Ethanol III	30 minutes
Xylene I	20 minutes
Xylene II	20 minutes
Xylene III	20 minutes
Paraffin I	10 minutes
Paraffin II	10 minutes
Paraffin III	10 minutes
Paraffin IV	20 minutes
Total	4 hours and 30 minutes

Vacuum shall be kept ON continuously.

4. Illustration of immunohistochemistry

: Procedure for prompt immunostaining for confirmatory test

Deparaffinization for 10 minutes

Washing in water 5 minutes

Autoclave processing 20 minutes at 121°C
(Soak in distilled water) (Required time 1.5 hours)

Applying 3% hydrogen peroxide water 5 minutes

Washing with PBS 5 minutes x 2 – 3 times

Reaction of primary antibody* 30 – 40 minutes at room temperature

Washing with PBS 5 minutes x 3 times

Reaction of secondary antibody** 30 minutes at room temperature

Washing with PBS 5 minutes x 2 – 3 times

Coloring with DAB 7 – 10 minutes

Washing with running water 5 minutes

Nucleus staining with hematoxylin 30 seconds

Washing with running water (warm water) 5 minutes

Dehydration/mounting 10 minutes

List of reagents and systems

(General reagents and equipment do not need to be from specific manufacturers as long as they are equivalent.)

	Reagent name	Manufacturer name	Standard	Unit
1	Sodium hydroxide	Wako	197-02125	500 g
2	Formalin (37.5%)	Wako	061-00411	3 L
3	Formic acid (98%)	Wako	066-00466	500 ml
4	Paraffin	Wako	164-13345	500 g
5	Alcohol	Sigma		4 L
6	DAKO PEN	DAKO		1 piece
7	Harris' hematoxylin	Mutoh	2002	500 ml
8	Mayer's hematoxylin	Mutoh	3001	500 ml
9	Eosin	Mutoh		500 ml
10	B103 or 44B1 anti-prion antibody	Fujirebio (44B1 is distributed)		
11	Envision+ kit (for mouse or rabbit)	DAKO		110 ml
12	Simple stain DAB kit	Histofine	415172	1 set
13	Hydrogen peroxide water	Wako	081-04215	500 ml
14	Normal rabbit blood serum	Any manufacturer		
15	Xylene for pathological purposes	Mutoh		15 Kg
16	Mount-quick (mounting medium)	Daido Sangyo		30 cc
17	Sodium dihydrogenphosphate (dihydrate)	Wako	199-02825	500 g
18	Disodium hydrogenphosphate · 12 H ₂ O	Wako	196-02835	500 g
19	Sodium chloride	Wako	191-01665	500 g

Appendix 2

	Equipment/system name	Manufacturer name	Standard	Unit
1	Disposable bench sheet	Wattmann	40 x 57 cm	50 sheets
2	Disposal blades for sectioning and cutting	Feather	No.130	A set of 50 blades
3	Plastic cassettes	Tissue-Tek	Procassette	1,000 pieces
4	Kimtowel	CRECIA	J-120	24 bundles
5	Container for formic acid processing	NALGENE	2118-0032	pieces
6	Silane-coated slides	Mutoh	1106	100 pieces
7	Cover glass	Mutoh	24 x 36	1,000 pieces
8	Microtome disposal blades	Feather	A35	50 pieces
9	Staining vat (for 20 pieces)	Matsunami		pieces
10	Staining basket (for 20 pieces)	Matsunami	B-20	pieces
11	Stainless vat		0.6L	pieces
12	Humidifying box	COSMO BIO	for 20 pieces	pieces
13	Latex gloves	Asahi Emas	DPG-350	Box (100 gloves)
14	Masks with plastic shield	Hogy Medical	FBM-281	50 pieces
15	Gowns	Hogy Medical	MGM-14	30 sets
16	Anti-cut gloves	Inai	LA132	10 gloves
17	Safety cabinet	Laboconco	LAAD-1300XA	
18	Slide washer	Juji Field	SW-4	
19	Autoclave 135°C	Tomy Seiko	KS-323	
20	Hood with HEPA filter	Oriental	Aura-700	
21	Vacuum with HEPA filter	Atomic	FC-111-A13	
22	Automatic embedding system	Sakura Finetek	ETV-150CV	
23	Paraffin stretcher	Sakura Finetek	PS-53	
24	Humidifier	Sakura Finetek	SMB-1	

(Supplementary) Procedure for preprocessing on anti-PrP antibody and pathological pieces

At present, anti-PrP peptide rabbit antibody and mouse monoclonal antibody are available for BSE confirmatory test. The former consists of B103 (Obihiro University of Agriculture and Veterinary Medicine) and T4 (National Institute of Infectious Diseases) and the latter consists of 44B1 and 43C5 (both from Obihiro University of Agriculture and Veterinary Medicine). It was discovered that the combination of preprocessings on antibody and specimen influence the results. As of now, it is desired that B103 is used as the primary antibody and 44B1 as the secondary after autoclaving in distilled water.

1. Preprocessing on sections

Preprocessing on the tissue sections after deparaffinization is conducted for the purpose of enhancement reaction with PrPSC (recovery of antigenicity or unmasking of antigens), and it is an inevitable process for BSE testing. The following 2 types of preprocessing methods were examined:

- 1) 20 minutes at 121°C in distilled water
- 2) 20 minutes at 121°C in 1mM HCl solution

Conventionally, proteinase processing was conducted additionally. However, it is not required in the current prompt fixing and embedding method.

For both of the above 2 methods, staining basket containing thesections was placed in a 400 ml stainless vat with a lid for autoclaving under the same conditions.

2. Antibody properties

- a) B103 rabbit antibody: Prepared using the 103·121 peptide of PrP protein N-end as the antigen. Conc.4.6 mg/ml
- b) T4 rabbit antibody: Prepared using the 221·239 peptide of PrP protein C-end as the antigen. Conc.0.6 mg/ml
 - ※ The above 2 rabbit antibodies are affinity purified antibodies.
- c) 44B1 mouse monoclonal antibody: Recognizes 155·231. Conc. 4 mg/ml
- d) 43C5 mouse monoclonal antibody: Recognizes 161·169. Conc. 4.6 mg/ml

3. Antibodies and methods of preprocessing

Antibody and dilution scale	1) DDW 20 minutes at 121°C		2) 1mM HCl 20 minutes at 121°C	
	Pos	Neg	Pos	Neg
B103 x500	+/-	-/-	3+< / +N	-/+D
T4 x1000	2+/-	-/-	3+> / ·	-/+·
44B1 x500	+/-	-/-	2+< / ·	-/-
43C x2000	2+ /+D	-/+	3+ /2+D	- /3+D

Pos: Positive control (2nd case in Hokkaido), Neg: Negative control (case with nonspecific observation: B026)

+/-: Signal positive (degree) / nonspecific reaction (degree)

The antibodies used here were manufactured by Fujirebio and the antibody concentration was 1 mg/ml.

4. Remarks

- 1) B103 is a monospecific polyclonal antibody, and it is expected to be used to recognize several antigen-determining groups. It is normally possible to detect PrPSC without problems under the conditions of 1). However, reactivity is rather weak. Weak nonspecific reaction may be observed in cell nuclei. Nonspecific staining of nuclei is especially strong under conditions of 2). Although staining under conditions of 2) is best, it accompanies nonspecific reactions and 1) is recommended as of now.
- 2) Best results are obtained under the conditions of 2) for T4 antibody. Nonspecific staining was observed in 3 cases in the past. Such nonspecific positive reaction was similar for B103 and 43C5 as well. However, it was not observed for 44B1. Furthermore, T4 does not remain in volume to be distributed.
- 3) While 44B1 can be used under any condition without nonspecific reactions, it has somewhat weak reaction (signal intensity). The staining intensity is stronger for 43C5 than for 44B1.
- 4) 43C5 shows nonspecific reactions in which staining is seen diffusively on the nerve cells and net such as olivary nucleus under any method.

ABATTOIR LAW

(Law No. 114, August 1, 1953)
As of February 27, 2004

(Purpose of Law)

Article 1. The purpose of this Law shall be to establish regulations and take other measures necessary from the viewpoint of public health in order to ensure the proper management of abattoirs and slaughter and dressing of livestock for human consumption, so as to protect people's health.

(Responsibility of the State, Prefectures and Cities with Health Centers)

Article 2. The State, prefectures and cities as designated by Cabinet Order pursuant to the provisions of Paragraph 1, Article 5 of the Community Health Law (Law No. 101 of 1947) (hereinafter referred to as "cities with health centers") shall, considering the actual situation of domestic animal production and the status of disease occurrence in animals, take necessary measures to ensure the proper processing of livestock for human consumption in order to prevent the occurrence of a health hazard.

(Definitions)

Article 3. 1. In this Law, "livestock" means cattle, horses, swine, sheep, and goats.

2. In this Law, "abattoir" means facilities established for the slaughter or dressing of livestock for human consumption.

3. In this Law, "general abattoir" means abattoirs of a scale allowing the slaughter or dressing of cattle or horses not less than one year of age or more than 10 animals each day as general practice.

4. In this Law, "simple abattoir" means abattoirs other than general abattoirs.

5. In this Law, "slaughterer" means persons carrying on the business of slaughtering or dressing animals.

(Permit for Establishment of Abattoirs)

Article 4. 1. General abattoirs or simple abattoirs shall not be established without permits from the Governor of the prefecture (in cases of cities with health centers, the Mayor of the city; the same applies hereinafter).

2. Persons wishing to receive permits, pursuant to the provisions of the preceding paragraph, shall submit a written application declaring construction, facilities, and other matters designated by Ministry of Health, Labour and Welfare Ordinance to the Governor of the prefecture.

3. Persons wishing to change the construction, facilities, or other matters designated by Ministry of Health, Labour and Welfare Ordinance for abattoirs established receiving permits pursuant to the provisions of Paragraph 1 shall notify the Governor of the prefecture in advance.

Article 5. 1. Governors of prefectures may, when there are applications for permits pursuant to the provisions of Paragraph 1 of the preceding article, refuse to grant permits of the same paragraph of said article when the locations of establishment of said abattoirs fall under any of the following items or when considering that the construction or facilities of said abattoirs do not comply with the standards of general abattoirs or simple abattoirs designated by Cabinet Order.

(1) Locations of crowded housing
(2) Locations liable to contaminate drinking water intended for public use

(3) Other locations considered by the governors of prefectures to be liable to cause a hazard in public health

2. Governors of prefectures may, when considering there is a need in public health, restrict the species and daily number of animals able to be processed as general practice in abattoirs in accordance to the scale of construction and facilities of said abattoirs receiving permits pursuant to the provisions of Paragraph 1 of the preceding article (hereinafter simply referred to as "abattoirs").

(Sanitation Control in Abattoirs)

Article 6. Owners and managers of abattoirs shall keep the inside and outside of the abattoirs continually clean, sufficiently treat waste, work to prevent the occurrence of and to expel rodents, insects, and the like, provide good sanitary control of the abattoirs pursuant to the standards prescribed by Ministry of Health, Labour and Welfare Ordinance, and take other measures necessary for public health.

(Sanitation Manager)

Article 7. 1. A sanitation manager shall be stationed at each abattoir by the manager of the abattoir (in cases of abattoirs without abattoir managers, the owner of the abattoir; the same applies hereinafter in this paragraph, Paragraph 6, the succeeding article and Item (5), Paragraph 1, Article 18) in order to ensure good sanitary control of the abattoir. *Provided, However,* That this does not apply to abattoirs managed by the abattoir managers who also serve as sanitary managers.

2. A sanitation manager shall, in order to prevent violation of this Law or any order or disposition under this Law in relation to sanitation control of the abattoir, supervise personnel engaged in sanitation control of the abattoir, manage the construction and facilities of the abattoir, and otherwise exercise cautions necessary for sanitation control of the abattoir.

3. A sanitation manager shall, in order to prevent violation of this Law or any order or disposition under this Law in relation to sanitation control of the abattoir, give

necessary advice on sanitation control of the abattoir to the owner or manager of the abattoir.

4. Owners and managers of abattoirs shall respect their sanitation managers' advice given in accordance with the provisions of the preceding paragraph.

5. No person may become a sanitation manager unless falling under one of the following items.

(1) Veterinarians

(2) Persons who successfully completed a course in veterinary medicine or livestock production at, and graduated from, a university under the School Education Law (Law No. 26 of 1947), a university under the former University Ordinance (Imperial Ordinance No. 388 of 1918), or a professional school under the former Professional School Ordinance (Imperial Ordinance No. 61 of 1903)

(3) Persons prescribed in Article 47 of the School Education Law, or those who are taken to have attained scholarship at the same level as or a higher level than said persons pursuant to designations by Ministry of Health, Labour and Welfare Ordinance and who have been engaged in the practice of sanitation control of abattoirs for at least 3 years as well as successfully completing a relevant course at a school held by the prefecture or the city with health centers

6. Upon appointment of a sanitation manager or upon becoming one, the manager of an abattoir shall notify the Governor of the prefecture, within 15 days following the date of such appointment or otherwise, of the sanitation manager's name or the fact that the abattoir manager has become a sanitation manager, as well as other matters designated by Ministry of Health, Labour and Welfare Ordinance. The same shall apply when wishing to change the sanitation manager.

7. The course subjects and other matters necessary for the course at school mentioned in Item (3), Paragraph 5 shall be designated by Ministry of Health, Labour and Welfare Ordinance.

Article 8. Governors of prefectures may order the manager of an abattoir to discharge the sanitation manager of the abattoir if he/she falls under any of the following items and is considered unfit to remain in office.

(1) Cases where the sanitation manager has violated this Law or any order or disposition under this Law

(2) Cases where the sanitation manager fails to perform the duties prescribed in Paragraph 2 of the preceding article

(Sanitary Control to be Taken by Slaughterers, Etc.)

Article 9. Slaughterers and other persons performing the slaughter or dressing of livestock (hereinafter referred to as "slaughterers, etc.") shall, when performing the slaughter or dressing of livestock in abattoirs, provide good sanitary control of the slaughter or dressing of livestock pursuant to the standards prescribed by Ministry of Health, Labour and Welfare Ordinance, and take other measures necessary for public health.

(Chief Sanitation Worker)

Article 10. 1. A chief sanitation worker shall be stationed at each abattoir by slaughterers, etc., in order to ensure good sanitary control of the slaughter or dressing of livestock. *Provided, However,* That this does not apply to abattoirs managed by the slaughterers, etc., who also serve as the chief sanitation worker.

2. The provisions from Paragraphs 2 through 7 of Article 2 and those of Article 8 shall be applied mutatis mutandis to chief sanitation workers. In this case, provisions necessary for technical adjustments of terms shall be prescribed by Cabinet Order.

(Restrictions on Refusal of Use, Etc., of Abattoirs)

Article 11. 1. Owners or managers of abattoirs shall not, without just reason, refuse the use of abattoirs for slaughter or dressing of livestock.

2. Slaughterers shall not, without just reason, refuse the slaughter or dressing of livestock.

(Abattoir Usage Fees and Slaughter-Dressing Fees)

Article 12. 1. Owners or managers of abattoirs, or slaughterers, shall receive the authorization of the Governor of the prefecture in advance for values designated as abattoir usage fees and slaughter-dressing fees. The same shall apply when wishing to change the amount of the authorized abattoir usage fees and slaughter-dressing fees.

2. Owners or managers of abattoirs, or slaughterers, shall not receive abattoir usage fees and slaughter-dressing fees exceeding the sums authorized pursuant to the preceding paragraph.

3. Owners or managers of abattoirs, or slaughterers, shall display on a readily visible location in the abattoirs the abattoir usage fees or slaughter-dressing fees authorized pursuant to the provisions of Paragraph 1.

(Slaughter or Dressing of Livestock)

Article 13. 1. No person shall slaughter livestock for human consumption in locations other than abattoirs. *Provided, However,* That this does not apply to the cases listed in the following.

(1) Cases of persons, other than persons carrying on meat retail businesses or other businesses handling meat designated by Ministry of Health, Labour and Welfare Ordinance, who notify the Governor of the prefecture in advance, pursuant to designations by Ministry of Health, Labour and Welfare Ordinance, of the slaughter of livestock (except cattle and horses over one year old) principally for consumption by said persons and their immediate family members

(2) Cases where livestock have been injured or have fallen into an incurable state due to unforeseen accidents and have to be immediately slaughtered

(3) Cases where livestock suffer from difficult delivery, puerperal paralysis, acute tympanites, or other diseases designated by Ministry of Health, Labour and Welfare Ordinance and have to be immediately slaughtered

- (4) Other cases designated by Cabinet Order
2. No person shall dress livestock for human consumption in locations other than abattoirs. *Provided, However,* That this does not apply to cases of dressing livestock slaughtered in locations other than abattoirs pursuant to the provisions of Items (1), (4), and (5) of the preceding paragraph.
3. Governors of prefectures may, when considering there is a need in public health, indicate to persons slaughtering or dressing livestock in locations other than abattoirs, pursuant to the provisions of the preceding two paragraphs, the location of slaughter or dressing, the method of processing meat, by products, and the method of treating waste.

(Inspection of Slaughter or Dressing of Livestock)

Article 14. 1. Abattoirs shall not slaughter livestock other than livestock passing inspections performed by the Governor of the prefecture.

2. Abattoirs shall not dress livestock other than livestock passing inspections performed by the Governor of the prefecture following slaughter.

3. The meat, viscera, blood, bones, and hide of livestock dressed in abattoirs shall not be transported outside abattoirs until after passing meat inspections performed by the Governor of the prefecture. *Provided, However,* That this does not apply to the cases falling under any of the following items.

(1) Cases where an official of the prefecture (in cases of cities with health centers, the city; the same applies hereinafter) carries part of the meat, viscera, blood, bones, or hide of dressed livestock outside the abattoir in cases where this is considered necessary for inspections mentioned in the first sentence of this paragraph

(2) Cases where hide of livestock is transported outside the abattoir with the permission of the Governor of the prefecture for inspections mentioned in the first sentence of this paragraph to be performed to determine the presence or absence of diseases designated by Ministry of Health, Labour and Welfare Ordinance, or other cases designated by Cabinet Order as those without sanitary concerns

4. Provisions of the preceding three paragraphs shall, except when the Governor of the prefecture considers there is no special need for inspection, apply *mutatis mutandis* to cases of slaughter or dressing of livestock in locations other than abattoirs pursuant to the provisions of Item (4), Paragraph 1 of the preceding article or the proviso to Paragraph 2 of the same article relating to this. In this case, references in the preceding paragraph to "outside abattoirs" shall be read as "outside locations where the dressing of livestock was performed."

5. Of affairs to be conducted under the authority of the Governors of prefectures prescribed in the preceding paragraphs of this article, those relating to inspections to determine the presence or absence of disease designated by Cabinet Order shall be, notwithstanding the provisions of the preceding paragraphs of this article, conducted by the Governors of prefectures and the Minister of Health, Labour

and Welfare pursuant to designations by Cabinet Order.

6. Inspections to be performed pursuant to the provisions of the preceding paragraphs of this article shall be performed to determine the presence or absence of the following.

(1) Infectious diseases among domestic animals designated in Paragraph 1, Article 2 of the Domestic Animal Infectious Disease Control Law (Law No. 166 of 1951) and notifiable infectious diseases designated in Paragraph 1, Article 4 of the same law

(2) Diseases other than those listed in the preceding item and designated by Ministry of Health, Labour and Welfare Ordinance

(3) Adherence of lubricating oil or other abnormalities designated by Ministry of Health, Labour and Welfare Ordinance

7. Other than those prescribed in the preceding paragraph, methods, procedures, and other necessary matters relating to inspections performed by the Governor of the prefecture and the Minister of Health, Labour and Welfare, pursuant to the provisions of Paragraphs 1 through 5 of this article, shall be designated by Cabinet Order.

8. Results of inspections performed by the Governor of the prefecture and the Minister of Health, Labour and Welfare, pursuant to the provisions of Paragraphs 1 through 5 of this article, may not be appealed under the Complaints Against Administrative Acts Inquiries Act (Law No. 160 of 1962).

(Prohibition of Transfer)

Article 15. No person shall accept, for sale as human food (including delivery other than sale to unspecified or numerous persons), meat or viscera of livestock dressed in locations other than abattoirs in violation of the provisions of Paragraph 2, Article 13 or meat or viscera of livestock transported out in violation of the provisions of Paragraph 3 of the preceding article (including cases of application *mutatis mutandis* in Paragraph 4 of said article and cases to which the provisions of Paragraph 5 of the same article are applied).

(Prohibition of Slaughter, Dressing, Etc.)

Article 16. Governors of prefectures may, when considering, as a result of inspections pursuant to the provisions of Article 14, that livestock suffer from disease or show abnormality and are not fit for use for human consumption, or when considering that said livestock or the slaughter or dressing of said livestock may cause transmission of disease, take the measures listed in the following to the extent necessary for public health.

(1) Prohibition of the slaughter or dressing of said animals

(2) Ordering of segregation of said animals, disinfection in the premises, or other measures to be taken by owners or managers of said animals, owners or managers of abattoirs, slaughterers, and other persons involved; or orders to officials concerned to take these measures

(3) Orders to owners or managers of meat and by

products of said animals to discard, or take other measures on the meat and by products of animals considered unfit for human consumption; or orders to officials concerned to take these measures

(Collection of Reports, Etc.)

Article 17. 1. Governors of prefectures and mayors of cities with health centers may, to the extent necessary for the enforcement of this Law, collect necessary reports from owners or managers of abattoirs, slaughterers or other persons involved; or order officials concerned to enter abattoirs, or offices, warehouses or other facilities of owners or managers of abattoirs, slaughterers or other persons involved to inspect equipment, accounting books, documents and other properties.

2. Officials performing spot inspections, pursuant to the provisions of the preceding paragraph, shall carry certificates showing their identification and shall display them upon request of persons involved.

3. The authority under the provisions of Paragraph 1 shall not be interpreted as being recognized for criminal investigations.

(Cancellation of Permits for Establishment of Abattoirs)

Article 18. Governors of prefectures may, in the cases listed in the following, cancel permits given pursuant to the provisions of Paragraph 1, Article 4 or order owners or managers of abattoirs to restrict or suspend usage of the facilities of said abattoirs for a designated period of time.

(1) When the construction and facilities of said abattoirs no longer comply with standards mentioned in the provisions of Paragraph 1, Article 5

(2) When abattoirs for which restrictions on the species and number of livestock have been designated, pursuant to the provisions of Paragraph 2, Article 5, perform slaughter or dressing of livestock not following those restrictions

(3) When simple abattoirs for which restrictions on the species and number of livestock have not been designated, pursuant to the provisions of Paragraph 2, Article 5, perform as general practice the slaughter or dressing of more than 10 animals per day or of cattle or horses over one year old

(4) When the owner or manager of the abattoir violates the provisions of Article 6 or Paragraph 1 or 6 of Article 7

(5) When the manager of the abattoir violates any order mentioned in the provisions of Article 8

2. Governors of prefectures may, in the cases listed in the following, order slaughterers, etc., to suspend the work of slaughter or dressing for a designated period of time or prohibit the performance of slaughter or dressing.

(1) When the slaughterer, etc., violates the provisions of Article 9 or Paragraph 1, Article 10, or Paragraph 6, Article 7 as applied mutatis mutandis in Paragraph 2, Article 10

(2) When the slaughterer, etc., violates any order

mentioned in the provision of Article 8 as applied mutatis mutandis in Paragraph 2, Article 10

(Inspectors)

Article 19. 1. Governors of prefectures shall appoint, from among officials of the prefectures, inspectors to engage in the administrative work of inspections prescribed in Article 14 and to perform the duties of officials concerned prescribed in Article 16 and Paragraph 1, Article 17 as well as duties involved in giving instructions in relation to ensuring the proper processing of livestock for human consumption.

2. Governors of prefectures shall cause inspectors to perform the administrative work or duties mentioned in the preceding paragraph pursuant to designations by the Prefectural Food Sanitation Monitoring and Guidance Program prescribed in Paragraph 1 of Article 24 of the Food Sanitation Law (Law No. 233 of 1947).

3. Necessary matters for qualifications of inspectors shall be designated by Cabinet Order.

(Request for Investigation, Etc., by Minister of Health, Labour and Welfare)

Article 20. When the Minister of Health, Labour and Welfare requests reports pursuant to the provisions of Article 60 of the Food Sanitation Law or otherwise considers it specifically necessary to prevent the occurrence of a food sanitation hazard, the Minister may request the Governor of the prefecture to perform inspections to be conducted pursuant to the provisions of Paragraphs 1 through 4 of Article 14, take measures pursuant to the provisions of Paragraph 1, Article 17, investigate into the cause of food poisoning, and report the results of investigations within a designated period of time.

(Collection of Public Opinions)

Article 21. 1. When the Minister of Health, Labour and Welfare intends to enact, amend or abolish any of the Ministry of Health, Labour and Welfare Ordinances mentioned in Articles 6 and 9, Item (3), Paragraph 1, Article 13 and Items (2) and (3), Paragraph 6, Article 14, or to draw up a proposal to enact, amend or abolish Cabinet Order mentioned in Paragraph 7 of said Article, the Minister shall publish the purpose, contents and other necessary matters and extensively collect public opinions. *Provided, However,* That this does not apply to cases of emergency to prevent the occurrence of a food sanitation hazard with no time allowed for extensive collection of public opinions.

2. In cases mentioned in the proviso to the preceding paragraph, the Minister of Health, Labour and Welfare shall extensively collect public opinions afterwards without delay.

(2. Communication and Cooperation)

Article 22. In the enforcement of this Law, the Minister of Health, Labour and Welfare and the Minister of Agriculture, Forestry and Fisheries shall keep in close contact and cooperate with each other regarding matters relating to ensuring the proper slaughter and dressing of

livestock for human consumption.

(Classification of Affairs)

Article 23. Affairs to be conducted by prefectures pursuant to the provisions of Paragraph 1, Article 17 shall fall under class 1 authorized affairs prescribed in Item (1), Paragraph 9, Article 2 of the Local Autonomy Law (Law No. 67 of 1947).

(Penal Provisions)

Article 24. Persons falling under any of the following items shall be punished by imprisonment of not more than three years or by a fine of not more than 3,000,000 yen.

- (1) Persons violating the provisions of Paragraph 1, Article 4
- (2) Persons violating the provisions of Paragraph 1 or 2 of Article 13
- (3) Persons violating any of the provisions of Paragraphs 1 through 3 of Article 14 (including cases of application mutatis mutandis in Paragraph 4 of said article and cases to which the provisions of Paragraph 5 of the same article are applied)

Article 25. Persons falling under any of the following items shall be punished by imprisonment of not more than one year or by a fine of not more than 1,000,000 yen.

- (1) Persons violating the provisions of Article 15
- (2) Persons violating any prohibition or order imposed pursuant to the provisions of Article 16 or persons refusing, obstructing, or evading implementation of duties of officials concerned pursuant to the provisions of Item (2) or (3) of said article
- (3) Persons violating any order imposed pursuant to the provisions of Paragraph 1, Article 18 or any order or prohibition imposed pursuant to the provisions of Paragraph 2 of said article

Article 26. Persons falling under any of the following items shall be punished by a fine of not more than 500,000 yen.

- (1) Persons failing to give notification pursuant to the provisions of Paragraph 6, Article 7 (including cases of application mutatis mutandis in Paragraph 2, Article 10) or giving false notification
- (2) Persons violating the provisions of Article 11
- (3) Persons receiving abattoirs usage fees or slaughter-dressing fees without authorization pursuant to the provisions of Paragraph 1, Article 12 or in violation of the provisions of Paragraph 2 of said article
- (4) Persons violating any instruction given pursuant to the provisions of Paragraph 3, Article 13
- (5) Persons failing to make reports pursuant to the provisions of Paragraph 1, Article 17, making false reports, or refusing, obstructing, or evading spot inspections of officials concerned

Article 27. If the president of a corporation or an agent, employee, or other worker of a corporation or person

commits any of the violations listed in the following items relating to work of such corporation or person, then in addition to the punishment of the violator, said corporation shall be punished by the fine prescribed in the relevant item below or said person by the fine prescribed in the article stipulated by the relevant item below, as the case may be.

- (1) Article 24: A fine of not more than 100,000,000 yen
- (2) Article 25 or the preceding article: The fine prescribed in the relevant article

SUPPLEMENTARY PROVISIONS

(Enforcement Date)

1. This Law shall come into force from the day of promulgation. *Provided, However,* That the provisions of Article 12 shall come into force after the passage of one month calculated from the day of promulgation.

(Abolition of Slaughter Law)

2. The slaughter Law (Law No. 32 of 1906) shall be abolished.

(Transitional Provisions Regarding Permit for Establishment of Abattoirs)

3. Of the current abattoirs that have been established with permits received pursuant to the former provisions of this Law at the time when this Law comes into force, those whose construction and facilities satisfy the standards for general abattoirs mentioned in the provisions of Paragraph 1, Article 5 and those which slaughter or dress more than 10 animals per day as general practice shall be deemed general abattoirs that have been established with permits pursuant to the provisions of this Law, and others shall be deemed simple abattoirs that have been established with permits pursuant to the provisions of this Law.

(Transitional Provisions Regarding Inspectors)

4. Persons who have been appointed as current inspectors at the time when this Law comes into force pursuant to the former provisions of this Law shall be deemed to have been appointed as inspectors pursuant to the current provisions of this Law.

(Transitional Provisions Regarding Penal Provisions)

5. The former penal provisions of this Law shall be applicable to acts conducted prior to the enforcement of this Law.

ABATTOIR LAW ENFORCEMENT ORDINANCE

(Cabinet Order No. 216, August 25, 1953)
As of February 27, 2004

(Standards of Construction and Facilities of General Abattoirs)

Article 1. Standards of construction and facilities of general abattoirs, pursuant to the provisions of Paragraph 1, Article 5 of the Abattoirs Law (hereinafter referred to as "the Law"), shall be as follows.

(1) Abattoirs shall have stockyard facilities, antemortem inspection facilities, processing rooms, cooling installations, inspection rooms, disinfection facilities, segregation facilities, waste treatment facilities, and, when meat (including viscera used for human consumption; the same applies in Item (5)) transactions are performed in the abattoirs and considered specifically necessary by the Governor of the prefecture (in cases of cities with health centers, the Mayor of the city; the same applies hereinafter), a trading room.

(2) Stockyard facilities shall be provided with partitions for each head of cattle or horse over one year of age and for appropriate numbers of other livestock, which are able to pen or hold animals and shall have floors constructed of impermeable materials (meaning stone, concrete, and other materials impermeable to blood and wastewater, the same applies hereinafter) which are provided with appropriate slopes and drainage gutters.

(3) Antemortem inspection facilities shall meet the following conditions.

a. Floors shall be constructed of impermeable materials.

b. Antemortem inspection facilities shall be equipped with installations necessary for weighing and retention of animals.

c. Antemortem inspection facilities shall be equipped with apparatus necessary for cleaning or disinfecting the fingers of, and instruments used by, persons engaged in inspections prescribed in Paragraph 1, Article 14 of the Law.

d. Apparatus necessary for cleaning or disinfecting shall be installed in number necessary for, and in positions appropriate for, carrying out measures prescribed in Paragraph 2, Article 8.

(4) Processing rooms shall meet the following conditions.

a. Processing rooms shall be partitioned into slaughter rooms, diseased animal slaughter rooms, viscera handling rooms, and hide handling rooms; and each room shall be provided with entrances and exits leading directly outside the processing rooms.

b. Floors shall be constructed of impermeable materials and shall be provided with appropriate slopes and drainage gutters.

c. Inside walls, except when constructed of impermeable materials, shall be covered with impermeable materials from the floor up to at least 1.2 meters.

d. Processing rooms shall be provided with windows allowing sufficient ventilation and natural lighting.

e. Processing rooms shall be equipped with viscera examination tables, viscera processing tables, viscera transportation equipment, meat hangers, and scales.

f. Processing rooms shall be equipped with

apparatus necessary for cleaning or disinfecting the fingers of, and instruments used by, persons who slaughter or dress animals and the persons engaged in inspections prescribed in Paragraph 2 or 3 of Article 14 of the Law.

g. Apparatus necessary for cleaning or disinfecting shall be installed in number necessary for, and in positions appropriate for, carrying out measures prescribed in Article 9 of the Law and measures prescribed in Paragraph 2, Article 8.

h. Processing rooms shall be equipped with hot water supplying installations allowing a sufficient supply of hot water needed for cleaning or disinfecting.

i. Processing rooms shall be provided with waterline connections allowing a sufficient supply of potable water.

(5) Cooling installations shall be capable of cooling meat thoroughly.

(6) Inspection rooms shall be equipped with inspection tables and other equipment needed for inspections and shall be provided with waterline connections.

(7) Disinfection facilities shall be provided with the facilities necessary for disinfection of those parts of animals which are considered to be a potential cause of disease transmission and shall have floors constructed of impermeable materials.

(8) Segregation facilities shall be provided with facilities able to disinfect the water and wastewater of segregated livestock and shall have floors constructed of impermeable materials.

(9) Waste treatment facilities shall meet the following conditions.

a. Waste treatment facilities shall have waste tanks and treatment facilities for blood and wastewater. *Provided, However,* That abattoirs flushing blood and wastewater directly into sewage systems with terminal treatment plants may omit treatment facilities for blood and wastewater.

b. Waste tanks shall be at an appropriate distance from processing rooms and trading rooms and shall be constructed of impermeable materials and be provided with appropriate covers.

c. Treatment facilities for blood and wastewater shall be at an appropriate distance from processing rooms and trading rooms and shall have blood and wastewater cleaning apparatus.

(10) Trading rooms shall meet the following conditions.

a. Floors shall be constructed of impermeable materials and shall be provided with appropriate slopes and drainage gutters.

b. Inside walls shall, except when constructed of impermeable materials, be covered with impermeable materials from the floor up to at least 1.2 meters.

c. Trading rooms shall be provided with windows allowing sufficient ventilation and natural lighting.

d. Trading rooms shall be provided with meat hangers and hanger rails.

e. Trading rooms shall be provided with waterline connections allowing a sufficient supply of potable water.

(11) Abattoirs shall have other construction and facilities designated by Ordinance of the prefecture (in cases of cities with health centers, the city; the same applies hereinafter).

(Standards of Construction and Facilities of Simple Abattoirs)

Article 2. Standards of structure and facilities of simple abattoirs, pursuant to the provisions of Paragraph 1, Article 5 of the Law, shall be as follows.

(1) Abattoirs shall have processing rooms, inspection areas, disinfection areas, waste treatment facilities, and the land necessary for performing antemortem inspections and segregation.

(2) Processing rooms shall meet the following conditions.

a. Processing rooms shall be provided with appropriate partitions allowing the individual handling of viscera and hide.

b. Floors shall be constructed of impermeable materials and shall be provided with appropriate slopes and drainage gutters.

c. Processing rooms shall be provided with windows allowing sufficient ventilation and natural lighting.

d. Processing rooms shall be equipped with viscera inspection tables, meat hangers, and scales.

e. Processing rooms shall be provided with waterline connections allowing a sufficient supply of potable water.

(3) Inspection areas shall be provided with inspection tables and waterline connections.

(4) Disinfection areas shall be provided with the facilities necessary for disinfection and shall have floors constructed of impermeable materials.

(5) Waste treatment facilities shall meet the following conditions.

a. Waste treatment facilities shall have waste tanks and wastewater tanks or treatment facilities for blood and wastewater. *Provided, However,* That abattoirs flushing blood and wastewater directly into sewage systems with terminal treatment plants may omit treatment facilities for blood and wastewater.

b. Waste tanks and wastewater tanks shall be at an appropriate distance from processing rooms and shall be constructed of impermeable materials and be provided with appropriate covers.

c. Treatment facilities of blood and wastewater shall be at an appropriate distance from processing rooms and have cleaning apparatus for blood and wastewater.

Provisions of the Law to be applied mutatis mutandis	Terms to be adjusted	Adjusted terms
Paragraph 2, Article 7	in relation to sanitation control of the abattoir	in relation to sanitation control of the slaughter or dressing of livestock
	sanitation control of the abattoir	slaughter or dressing of livestock at the abattoir
	manage the construction and facilities of the abattoir, and otherwise exercise cautions necessary for sanitation control of the abattoir	and otherwise exercise cautions necessary for sanitation control of the slaughter or dressing of livestock at the abattoir
Paragraph 3, Article 7	in relation to sanitation control of the abattoir	in relation to sanitation control of the slaughter or dressing of livestock
	sanitation control of the abattoir	sanitation control of the slaughter or dressing of livestock at the abattoir
	owner or manager of the abattoir	slaughterers, etc.
Paragraph 4, Article 7	Owners and managers of abattoirs	Slaughterers, etc.
Item (3), Paragraph 5, Article 7	sanitation control of abattoirs	slaughter or dressing of livestock
Paragraph 6, Article 7	the manager of an abattoir	a slaughterer, etc.
Article 8	the manager of an abattoir	a slaughterer, etc.
Item (2), Article 8	Paragraph 2 of the preceding article	Paragraph 2 of the preceding article as applied mutatis mutandis pursuant to the provisions of Paragraph 2, Article 10

(Adjustment of Provisions of the Law Applied Mutatis Mutandis to Chief Sanitation Workers)

Article 3. The technical adjustments of terms referred to in the provisions of Paragraphs 2 through 6 of Article 7 and Article 8 of the Law in cases where these provisions are applied mutatis mutandis to chief sanitation workers pursuant to Paragraph 2, Article 10 of the Law shall be as prescribed in the following table.

(Cases of Slaughter of Livestock Permitted in Locations Other Than Abattoirs)

Article 4. Cases where slaughter of livestock for human consumption is permitted in locations other than abattoirs, pursuant to the provisions of Item (4), Paragraph 1, Article 13 of the Law, shall be the cases listed in the following.

- (1) Cases where the destruction of abattoirs or damage of facilities by disasters or other accidents make slaughter in locations other than abattoirs unavoidable.
- (2) Cases where islands or other geographical conditions make slaughter in locations other than abattoirs unavoidable: where those regions are designated by the Governor of the prefecture or where the permit of the Governor of the prefecture has been received for the slaughter of livestock.

(Exceptions to Prohibition of Transporting Outside Abattoirs)

Article 5. 1. The cases to be prescribed by Cabinet Order mentioned in Item (2), Paragraph 3 of Article 14 of the Law shall be as follows.

(1) Cases where the inspections mentioned in the first sentence of Paragraph 3, Article 14 of the Law to determine the presence or absence of disease designated by Ministry of Health, Labour and Welfare Ordinance mentioned in Item (2) of said paragraph (referred to as the "post-dressing inspections in the succeeding item and Item (3)) are performed and where cattle hide is transported outside the abattoir as materials for leather with the permission of the Governor of the prefecture

(2) Cases where the post-dressing inspections are performed and where cattle ovaries are transported outside the abattoir for the purpose of improvement or breeding of cattle (including cases where such materials are used for research purposes) with the permission of the Governor of the prefecture

(3) Cases where the post-dressing inspections are performed and where the owner or manager of meat, viscera, blood, bones, or hide of livestock (hereinafter referred to as "livestock meat, etc." in this item through Item (5)) transports all or part of such livestock meat, etc., outside the abattoir for incineration with the permission of the Governor of the prefecture

(4) Cases where a food sanitation inspector samples part of livestock meat, etc., pursuant to the provisions of Paragraph 1, Article 28 of the Food Sanitation Law (Law No. 233 of 1947)

(5) Cases where an animal quarantine officer or a livestock disease prevention and control officer samples or collects part of livestock meat, etc., pursuant to the provisions of Paragraph 1, Article 51 of the Domestic Animal Infectious Disease Control Law (Law No. 166 of 1951) and transports outside the abattoir

2. The standards for giving the permission mentioned in Items (1) through (3) of the preceding article shall be prescribed by Ministry of Health, Labour and Welfare Ordinance.

3. The permissions mentioned in Items (1) through (3), Paragraph 1 may be given on certain conditions to the extent

necessary for public health.

(Inspections by Governors of Prefectures and Minister of Health, Labour and Welfare)

Article 6. 1. Diseases to be designated by Cabinet Order mentioned in Paragraph 5, Article 14 of the Law shall be transmissible spongiform encephalopathy in cattle, sheep and goats.

2. The affairs to be conducted by Governors of prefectures pursuant to the provisions of Paragraph 5, Article 14 of the Law shall be as follows.

(1) Inspections to be performed pursuant to the provisions of Paragraphs 1 and 2 of Article 14 of the Law (including cases of application mutatis mutandis in Paragraph 4 of said article) to determine the presence of absence of the diseases designated in the preceding paragraph

(2) Of the inspections to be performed pursuant to the provisions of Paragraph 3, Article 14 of the Law (including cases of application mutatis mutandis in Paragraph 4 of said article; the same applies in the succeeding paragraph) to determine the presence or absence of diseases designated by Ministry of Health, Labour and Welfare Ordinance from among those designated in the preceding paragraph, inspections performed using simple methods to detect materials that have to be submitted for confirmation testing (meaning testing performed using sophisticated methods to confirm the presence of disease; hereinafter the same applies)

3. The affairs to be conducted by the Minister of Health, Labour and Welfare pursuant to the provisions of Paragraph 5, Article 14 of the Law shall be the inspections to be performed pursuant to the provisions of Paragraph 3, Article 14 of the Law to determine the presence or absence of the diseases designated in Paragraph 1 (limited to confirmation testing in cases of inspections to determine the presence or absence of the diseases to be designated by Ministry of Health, Labour and Welfare Ordinance mentioned in Item (2) of the preceding paragraph).

4. In cases of prefectures considered by the Minister of Health, Labour and Welfare to possess sufficient technical capabilities for properly performing confirmation testing (excluding portions relating to judgment of the results of such confirmation testing; the same applies hereinafter in this paragraph), the Governors of such prefectures may perform the confirmation testing to be performed by said Minister pursuant to the provisions of the preceding paragraph, notwithstanding the provisions of the preceding two paragraphs.

(Applications for Inspections)

Article 7. Persons wishing to undergo inspections, pursuant to the provisions of Article 14 of the Law, shall submit a written application declaring the items designated by Ministry of Health, Labour and Welfare Ordinance to the Governor of the prefecture.

(Method of Inspection)

Article 8. 1. Inspections pursuant to the provisions of Article 14 of the Law shall be performed by visual diagnosis, thermometry, manual examination, dissection examination, microscopic examination, and other necessary methods.

2. Persons who engage in inspections prescribed in the preceding paragraph shall use clean instruments, wash or disinfect their fingers, instruments, etc., as the need arises, and take other measures necessary for public hygiene.

(Inspection Stamp)

Article 9. Governors of prefectures shall, when performing inspections pursuant to the provisions of Paragraph 3, Article 14 of the Law (including cases where the Governor of a prefecture and the Minister of Health, Labour and Welfare perform inspections pursuant to the provisions of Paragraph 5 of said Article), affix inspection stamps on meat, viscera, and hide passing the inspections pursuant to designations by Ministry of Health, Labour and Welfare Ordinance.

(Qualifications of Inspectors)

Article 10. Inspectors, prescribed in Paragraph 1, Article 19 of the Law, shall be veterinarians.

SUPPLEMENTARY PROVISIONS

(Enforcement Date)

1. This Cabinet Order shall come into force from the date of enforcement of the Law for Partial Amendment to the Food Sanitation Law, Etc. (August 29, 2003)

ABATTOIR LAW ENFORCEMENT REGULATION

(Ministry of Health and Welfare Ordinance
No. 44, September 28, 1953)
As of February 27, 2004

(Matters to be Declared on Written Applications for Establishment of Abattoirs)

Article 1. 1. The matters to be declared on the written applications, pursuant to the provisions of Paragraph 2, Article 4 of the Abattoir Law (Law No. 114 of 1953; hereinafter referred to as "the Law"), shall, other than matters prescribed in said paragraph of said article, be as follows.

(1) Address, name, and date of birth of applicant (for corporations, name, location of principal offices, name of president, and copy of articles of incorporation or act of endowment)

(2) Name and location of abattoirs

(3) Classification as to general abattoirs or simple abattoirs

(4) Species and daily number of livestock processed

(5) In cases where said abattoirs will carry out transactions of meat, an outline of such transactions

2. Written applications prescribed in the preceding paragraph shall be accompanied by working regulations declaring the outline of the management and business operation of said abattoirs or documents declaring items based on this.

(Matters to be Notified in Changes in Abattoirs)

Article 2. The matters to be notified, pursuant to the provisions of Paragraph 3, Article 4 of the Law, shall, other than the matters prescribed in said paragraph of said article, be principal matters of those listed in the items (except Item (3)) of Paragraph 1 of the preceding article and those declared in attached documents prescribed in Paragraph 2 of said article.

(Sanitation Control of Abattoirs)

Article 3. The standards prescribed by Ministry of Health, Labour and Welfare Ordinance mentioned in Article 6 of the Law shall be as follows.

(1) By giving proper cleaning, manage the abattoir without trouble from the viewpoint of sanitation.

(2) The abattoir shall be arranged and put in order, and unnecessary things shall be kept out of the abattoir.

(3) In cases where there are ruptures or failures in floors, inner walls, ceilings, windows or doors, they shall be repaired or mended without delay.

(4) The abattoir shall be fully ventilated to remove foul odor and excessive humidity.

(5) Necessary illumination shall be secured by means of natural illumination or lighting equipment.

(6) In cases where ventilation equipment is installed, provide proper maintenance of said equipment.

(7) Sanitation control of water supply installation shall be done in accordance with the following.

a. In the case where water other than that supplied from the city water service or exclusive waterworks prescribed in the Waterworks Law (Law No. 177, 1957) is used, conduct the quality test of water once or more in a year (every time when the quality of water may have changed because of the contamination of the water source, etc., due to a disaster, etc.), and preserve the paper certifying the result of the test for a year after the day of the test. In cases where the water has been proved to be undrinkable, immediately receive instructions from the Governor of the prefecture (in cases of cities with health centers, the Mayor; the same applies hereinafter), and take appropriate measures.

b. In cases where disinfecting equipment or water purifying equipment is used, certify every day that the said equipment is working normally. In these cases, keep papers recording necessary matters including the date of the confirmation, the result of the confirmation and the person who confirmed for a year after the day of the confirmation.

c. In cases where water storage tanks are used, inspect and clean them periodically.

(8) In cases where refrigerating installations are used, provide proper maintenance of said installations so that they can keep the temperature of dressed carcasses (which are made by cutting off the head, fore-limbs and hind-limbs, and the tail of a slaughtered animal and treating pursuant to Items (5), (6) and (7) of the succeeding article) or viscera for human consumption at 10 degrees centigrade or lower. In these cases, measure the temperature inside the refrigerating installations once before the beginning of daily hours of operation and once or more during the hours, and keep a record of necessary matters including the day and time of the measurement, the temperature and the person who did the measurement.

(9) Dressed carcasses reserved in the test prescribed in Paragraph 3, Article 14 of the Law shall be separated from other dressed carcasses and controlled sanitarilly.

(10) Sanitation control of stockyard facilities and antemortem inspection facilities shall be performed in accordance with the following.

a. Properly dispose of feces, etc., of livestock and wash the area.

b. Livestock with large amounts of feces, etc., on it shall be washed.

(11) A chamber in which hide of livestock is treated shall be kept clean.

(12) In cases where waste reservoirs and disposal facilities of blood and waste water are used, provide proper maintenance of said facilities. Sludge, etc., coming from said facilities shall be disposed of so that it will cause no sanitary trouble. In this case, the record of necessary matters including the day and the method of disposal, and the person who did the disposal shall be preserved for a year after the day of the disposal.

(13) Drainage ditches shall be cleaned so that solid matter will not flow away and waste water will be properly drained out, and shall be immediately repaired when damaged.

(14) Cleaning and disinfecting inside an abattoir shall be performed in accordance with the following.

a. In order to wash parts with blood or fat, etc., attached, use hot water.

b. Washing after the end of work shall be done using detergents.

c. Washing other than mentioned in a. and b. shall be done using sufficient amounts of water, hot water or detergents.

d. Disinfection shall be performed using hot water having a temperature of 83 degrees centigrade or higher, or disinfectants.

(15) Sanitary control of machinery and equipment shall be performed in accordance with the following.

a. Machinery and equipment shall be washed or disinfected after the end of work.

b. Disinfection of machinery and equipment directly contacting carcasses (animals after being slaughtered, other than dressed carcasses, the same applies hereafter) or dressed carcasses, including knives, powered skinning knives, saws, ligating instruments and others used for slaughtering or dressing of livestock shall be performed

using hot water having a temperature of 83 degrees centigrade or higher.

c. Machinery and equipment, and disassembled parts of them shall be kept sanitarilly each in a fixed place.

d. Machinery and equipment shall be checked at regular intervals and when there are failures or ruptures, repair or fix them immediately and maintain them so that they can be used properly all the time.

e. Measuring instruments including thermometers, manometers and flow meters shall be checked at regular intervals as to their accuracy and when there are failures or abnormalities, make repairs without delay.

(16) Sanitary control of non-edible parts shall be performed in accordance with the following.

a. Non-edible parts (excluding the parts listed in Attached Table 1), materials discarded pursuant to the provisions of Item (3), Article 16, materials discarded pursuant to the provisions of Item (4) of said article, the parts listed in Attached Table 1 and other waste shall be placed in containers used exclusively for this purpose and bearing an indication of the type of the discarded materials therein, removed from the processing room, and shall be disposed of in a manner that will prevent them from causing sanitary trouble, such as by incineration in an incinerator. In this case, with regard to disposal of materials discarded pursuant to the provisions of Item (4) of said article, preserve the record of necessary matters including the date, methods and performer of the disposal for a year after the date of such treatment.

b. Containers mentioned in a. shall be washed and disinfected at a designated place after completion of the work.

(17) Control of rats, insects and the like shall be performed in accordance with the following.

a. Windows and doorways without controlling equipment against rats and insects shall not be left open.

b. Controlling equipment against rats and insects including rat screens and insect screens shall be checked as to its function and shall be repaired if necessary.

c. In order to prevent invasion of insects by means of containers carried into processing rooms, those containers shall be checked at the time of the receipt, and containers which have become useless shall be removed from the processing rooms and shall be disposed of in a manner that will prevent them from causing sanitary trouble, such as by incineration in an incinerator.

d. Perform exterminating operations at regular intervals. In this case, preserve the record of necessary matters including the date and methods of the exterminating operation and persons who exercised the operation for a year after the date of such exterminating operation.

(18) Installations for washing hands shall be provided with cleaning and disinfecting liquid in a condition enabling use at any time.

(19) Lavatories shall be kept clean and disinfected at regular intervals.

(20) Instruments for cleaning shall be kept in a designated place.

(21) Handling of detergents, disinfectants and agents

including rodenticides and insecticides shall be performed in accordance with the following.

a. They shall be stored in designated places other than processing rooms and places for keeping dressed carcasses.

b. Use agents that conform to the objective using proper methods.

c. Prevent agents from contaminating carcasses, dressed carcasses and viscera for human consumption.

d. In cases where containers of detergents, disinfectants, etc., have been newly unsealed, preserve the record of necessary matters including the date of unsealing, the name of the agent unsealed, and the name of the person who unsealed the agent, for a year after the unsealing of the agent.

e. In cases where rodenticides and insecticides have been used, preserve the record of necessary matters including the date of the use, the name and used quantity of the agent used, and the name of the person who used the agent, for a year after the date of the use.

(22) Provide control in accordance with the following to ensure that measures prescribed in the preceding items in this paragraph shall be taken properly.

a. Draw up a document recording necessary matters in order to carry out the measures properly and systematically.

b. Cause the sanitation manager prescribed in Paragraph 1, Article 7 of the Law (hereinafter referred to as the "sanitation manager") to check that the measures have been properly carried out pursuant to the document mentioned in a. *Provided, However,* That in cases of abattoirs whose manager or owner serves as the sanitation manager of the abattoir pursuant to the provisions of said paragraph, the manager or owner of the abattoir shall perform the duties of checking.

2. The sanitation manager shall report results of the checking mentioned in b. No. (22) of the preceding paragraph to the owner or manager of the abattoir. *Provided, However,* That this does not apply to cases where the manager or owner of the abattoir serves as the sanitation manager of the abattoir pursuant to the provisions of Paragraph 1, Article 7 of the Law.

3. In the application of a., Item (16), Paragraph 1 to the parts listed in Attached Table 1, references in a. of said item to "such as by incineration in an incinerator" shall be read as "by incineration in an incinerator except in cases falling under the proviso to Paragraph 2, Article 7 of the Law on Special Measures Against Bovine Spongiform Encephalopathy (Law No. 70 of 2002)."

(Qualifications of Sanitation Managers)

Article 4. Persons who are taken to have attained scholarship at the same level as or a higher level than those prescribed in Article 47 of the School Education Law (Law No. 26 of 1947), described in Item (3), Paragraph 5, Article 7 of the Law, shall be as follows.

(1) Those who successfully completed a senior course at a national school under the former National School

Ordinance (Imperial Ordinance No. 148 of 1941)

(2) Those who completed a two-year course at a secondary school under the former Secondary School Ordinance (Imperial Ordinance No. 36 of 1943)

(3) Those who successfully completed the second year at an attached junior high school or an attached girls' senior high school under the former Teacher Education Ordinance (Imperial Ordinance No. 109 of 1943)

(4) Those who successfully completed the second year at a junior high school for the deaf under the former Schools for Blind and School for Deaf Ordinance (Imperial Ordinance No. 375 of 1923)

(5) Those who successfully completed the second year of an ordinary course at a high school under the former High School Ordinance (Imperial Ordinance No. 389 of 1918)

(6) Those who successfully completed a course of an ordinary course at a youth school under the former Youth School Ordinance (Imperial Ordinance No. 254 of 1939)

(7) Those who successfully completed a senior course at a national school, or completed a two-year course at a junior high school, or treated as those prescribed in Item (5), pursuant to the provisions of Articles 1 through 3 and Article 7 of the Regulations Regarding the Entrance and Transfer of School Children, Children, Graduates, Etc., of Schools in Regions Outside the Mainland to Other Schools (Ministry of Education, Science, Sports and Culture Ordinance No. 63 of 1943)

(8) Those who graduated from a seamen's training center under the former Seamen's Training Center Establishment Ordinance (Imperial Ordinance No. 458 of 1939)

(9) Other than those listed in the preceding items of this article, those who are considered by the Minister of Health, Labour and Welfare to have attained scholarship at the same level as or a higher level than persons prescribed in Article 47 of the School Education Law in relation to the qualifications of sanitation managers

(Matters to be Notified Regarding Sanitation Managers)

Article 5. 1. The matters designated by Ministry of Health, Labour and Welfare Ordinance mentioned in Paragraph 6, Article 7 of the Law shall be as follows.

(1) Name and address of the notifier and, in cases of corporations, name of the president thereof

(2) Name and address of the abattoir

(3) Name, address and date of birth of the sanitation manager

(4) The fact that the sanitation manager falls under one of the items of Paragraph 5, Article 7 of the Law

(5) Date of appointment or change of the sanitation manager

2. The notification pursuant to the preceding paragraph shall be accompanied by written evidence proving that the sanitation manager falls under one of the items of Paragraph 5, Article 7 of the Law.

(Course at School for Sanitation Manager)

Article 6. The course at a school designated by Ministry of Health, Labour and Welfare mentioned in Paragraph 7, Article 7 of the Law shall meet all of the following requirements.

(1) The subjects listed in the upper section of Attached Table 2 shall be taught for the respective numbers of hours listed in the lower section of said table and the school is held for at least 3 days.

(2) Instructors shall be persons who are in charge of subjects equivalent to those listed in the upper section of Attached Table 2 at universities under the School Education Law; persons engaged in testing services related to food sanitation administration or food sanitation for the State, prefectures, or cities or special wards with health centers; or persons whose knowledge and experience is considered equivalent to that of the aforementioned persons.

(3) The qualifications of participants to the school shall be that they are persons who graduated from a junior high school under the School Education Law or a school equivalent thereto, or successfully completed a junior course at a secondary education school, or fall under any of the items of Article 4, AND who have been engaged in the practice of sanitation control of abattoirs for at least 3 years.

(4) Participants' successful completion of the course shall be properly acknowledged by means of examinations or otherwise at the completion of the school.

(Sanitary Measures to be Taken by Slaughterers, Etc.)

Article 7. 1. The standards prescribed by Ministry of Health, Labour and Welfare mentioned in Article 9 of the Law shall be as follows.

(1) In processing rooms, properly treat blood and contents of the digestive tracts of livestock and wash the processing room. In this case, prevent the contamination of carcasses, dressed carcasses and viscera for human consumption by the spread washing water.

(2) In cases where gloves are used on the occasion of slaughter or dressing of livestock, use those gloves whose parts directly contacting the livestock are not made of substances difficult to wash and disinfect, such as textile products.

(3) Bleeding shall be performed in accordance with the following.

a. Prevent contamination of living animal or other carcasses by the blood released by the bleeding.

b. With regard to cattle, sheep and goats, ligate or block the esophagus in a place near the rumen in order to prevent the contents of digestive tracts from leaking out after the bleeding.

c. When fingers (in cases where gloves are used, the gloves; the same applies hereinafter in this paragraph) get contaminated by blood, etc., released by the bleeding, wash the fingers each time using detergents.

d. Machinery and instruments which directly contact carcasses including knives and ligating instruments

shall be, each time an animal is processed (in cases where the machinery or equipment is contaminated by contacting hide, etc., on all such occasions; the same applies hereinafter in the succeeding item and Item (5)), washed and disinfected using hot water having a temperature of 83 degrees centigrade or higher.

(4) Processing of head of livestock shall be done in accordance with the following.

a. Horns shall be removed together with outer skin in order to prevent contamination caused by remaining hide near the amputated part.

b. Prevent the skinned head from being contaminated by hide or from contact with the floor or inner walls.

c. When washing skinned heads, prevent the scattered washing water from contaminating other carcasses.

d. When fingers have been contaminated by hide, etc., wash the fingers each time using detergents.

e. Machinery and instruments that directly contact carcasses, such as knives and saws, shall be washed and disinfected using hot water having a temperature of 83 degrees centigrade or higher, each time an animal has been processed.

(5) Skinning of carcasses shall be performed according to the following.

a. In order to prevent contamination by hair, etc., of the animal, make the minimum incision necessary, then disinfect the knife, and cut the skin from the inside toward the outside while keeping the edge of the knife toward the operator.

b. Prevent the skinned part from being contaminated by hide.

c. In cases where the skinned part has been contaminated by hide, cut off the contaminated part entirely.

d. When processing parts surrounding the anus of cattle, sheep and goats, ligate the rectum near the anus to prevent the contents of the digestive tracts from leaking out, and at the same time prevent the contamination of the carcass by the anal part.

e. In cases where a skinned part has been contaminated by the contents of the digestive tracts, promptly prevent the other parts from being contaminated, and entirely cut off the contaminated part.

f. When fingers have been contaminated by hide, etc., wash the fingers each time using detergents.

g. Machinery and instruments which directly contact carcasses, such as knives, powered skinning knives and ligating instruments, shall be washed and disinfected using hot water having a temperature of 83 degrees centigrade or higher, each time an animal has been processed.

(6) Excision of breasts should be performed in accordance with the following.

a. Prevent the contents of the breasts from leaking out.

b. In cases where a skinned part has been contaminated by the contents of the breasts, promptly prevent the other parts from being contaminated, and entirely cut off the contaminated part.

c. When fingers have been contaminated by the contents of the breasts, wash the fingers each time using detergents.

d. Machinery and instruments which directly contact carcasses, such as knives, shall be washed and disinfected using hot water having a temperature of 83 degrees centigrade or higher, each time an animal has been processed (in cases where they are contaminated by the contents of breasts, on all such occasions).

(7) Extraction of viscera shall be performed in accordance with the following.

a. Prevent the carcass from being contaminated by the contents of the digestive tracts.

b. Prevent the viscera from being contaminated through contact with the floor, inner walls, boots, etc.

c. In cases where a skinned part has been contaminated by the contents of the digestive tracts, promptly prevent the other parts from being contaminated, and entirely cut off the contaminated part.

d. When fingers have been contaminated by the contents of the digestive tracts, wash the fingers each time using detergents.

e. Machinery and instruments that directly contact carcasses, such as knives and saws shall be washed and disinfected using hot water having a temperature of 83 degrees centigrade or higher, each time an animal has been processed (in cases where they are contaminated by the contents of the digestive tracts, on all such occasions).

(8) Halving (the process of cutting the dressed carcass right and left along the vertebral column) shall be performed in accordance with the following.

a. Prevent the dressed carcass from being contaminated through contact with the floor, inner walls, boots or the platform.

b. Saws used in the process shall be washed and disinfected using hot water having a temperature of 83 degrees centigrade or higher, each time an animal has been processed.

(9) Washing of dressed carcasses shall be performed in accordance with the following.

a. Before the washing, ascertain whether there is contamination by hair of livestock, contents of the digestive tracts and the like, and when there is such contamination, cut off the contaminated part entirely.

b. Wash using a sufficient amount of water.

c. Prevent the dressed carcasses from being contaminated by spread washing water.

d. Drain the washing water thoroughly.

(10) Treat dressed carcasses and viscera for human consumption preventing them from contacting the floor and inner walls.

(11) Processing of viscera shall be performed in accordance with the following.

a. The digestive tracts shall be treated separately in order to prevent the other viscera from being contaminated by the contents of the digestive tracts.

b. Prevent the viscera for human consumption from being contaminated through contact with the floor and

inner walls.

c. When treating the digestive tracts, remove the contents of the digestive tracts in order to prevent contamination by the contents, and wash said digestive tracts thoroughly.

d. In cases where the viscera-treating board has been contaminated by the contents of the digestive tracts, wash and disinfect the board on all such occasions.

(12) Refrigerate dressed carcasses or viscera for human consumption so that their temperature falls to 10 degrees centigrade or lower.

(13) Dressed carcasses reserved in the test prescribed in Paragraph 3, Article 10 of the Law shall be stored separately from other dressed carcasses.

(14) Hide shall be stored in a way preventing it from contacting dressed carcasses or viscera for human consumption.

(15) The parts listed in Attached Table 1 shall be disposed of in a manner that will prevent them from contaminating dressed carcasses and viscera for human consumption.

2. Slaughterers, etc., shall provide control in accordance with the following to ensure that the measures prescribed in the items of the preceding paragraph shall be taken properly.

(1) Draw up a document recording necessary matters in order to carry out the measures properly and systematically.

(2) Cause the chief sanitation worker prescribed in Paragraph 1, Article 10 of the Law (hereinafter referred to as the "chief sanitation worker") to check that the measures have been properly carried out pursuant to the document mentioned in the preceding item. *Provided, However,* That in cases of abattoirs whose manager or owner serves as the chief sanitation worker of the abattoir pursuant to the provisions of said paragraph, the manager or owner of the abattoir shall perform the duties of checking.

3. The chief sanitation worker (in cases of abattoirs for which the slaughterer, etc., serves as the chief sanitation worker pursuant to the provisions of Paragraph 1, Article 10 of the Law, the slaughterer, etc.) shall endeavor to provide education regarding sanitary methods of slaughtering or dressing livestock to those who slaughter or dress livestock.

(Application Mutatis Mutandis to Chief Sanitation Workers)

Article 8. The provisions of Articles 4 through 6 shall be applied mutatis mutandis to chief sanitation workers. In this case, the references in Item (4), Paragraph 1 and Paragraph 2 of Article 5 to "the items of Paragraph 5, Article 7 of the Law" shall be read as "the items of Paragraph 5, Article 7 of the Law as applied mutatis mutandis in the provisions of Paragraph 2, Article 10 of the Law."

(Range of Businesses Handling Meat)

Article 9. The businesses handling meat, prescribed in Item (1), Paragraph 1, Article 13 of the Law, shall, other than those prescribed in said item, be as follows.

(1) Meat processing businesses

(2) Meat product manufacturing businesses

- (3) Restaurant businesses
- (4) Daily-dish manufacturing businesses

(Notification of Private Slaughter)

Article 10. The notification pursuant to the provisions of Item (1), Paragraph 1, Article 13 of the Law shall be given of the following matters.

- (1) Address, name, date of birth, and profession of the notifier
- (2) Date and time of desired slaughter
- (3) Outline of the location of desired slaughter, and its surroundings
- (4) Species, sex, age (when not known, estimated age), characteristics, and weight of livestock desired to be slaughtered
- (5) Range of persons for whom meat is intended for consumption
- (6) When intended for consumption by persons other than one's self and immediate family members, declaration to that effect and the quantity

(Diseases Mentioned in Item (2), Paragraph 3, Article 14 of the Law)

Article 11. The disease designated by Ministry of Health, Labour and Welfare mentioned in Item (2), Paragraph 3, Article 14 of the Law shall be transmissible spongiform encephalopathy in cattle.

(Standards for Giving Permission for Transporting Materials Outside Abattoirs)

Article 12. 1. The standards for giving the permission mentioned in Item (1), Paragraph 1, Article 5 of the Abattoir Law Enforcement Ordinance (Cabinet Order 216 of 1953; hereinafter referred to as the "Ordinance") shall be as follows.

- (1) Measures have properly been taken to identify the individual cattle from which the cattle hide transported outside the abattoir derives from, until after completion of the post-dressing inspections (the post-dressing inspections prescribed in Item (1), Paragraph 1, Article 5 of the Ordinance; hereinafter the same applies).
- (2) Measures have properly been taken to prevent a loss of cattle hide transported outside the abattoir until after completion of the post-dressing inspections.
- (3) The facilities at which cattle hide transported outside the abattoir is to be preserved (including preservation in salt; the same applies hereinafter in this paragraph) constitute a rendering plant prescribed in Paragraph 2, Article 1 of the Rendering Plant Control Law (Law No. 140 of 1948) or are facilities for preservation of livestock hide prescribed in Article 8 of said law, AND are capable of properly preserving said cattle hide until after completion of the post-dressing inspection.
- (4) Measures have been taken, by the manager of the abattoir (in cases of abattoirs without managers, the owner of the abattoir; the same applies hereinafter in this article) from which cattle hide is to be transported, to properly record the name and contact information of the

person or entity transporting the cattle hide outside the abattoir, the name and contact information of the facilities at which the cattle hide is preserved, and other information necessary to ensure a good control system.

(5) Measures have been taken, at the facilities in which cattle hide transported outside the abattoir is to be preserved, to properly record the name and contact information of the person or entity transporting the cattle hide from the abattoir, the name and contact information of the abattoir from which the cattle hide was transported, and other information necessary to ensure a good control system.

2. The standards for giving the permission mentioned in Item (2), Paragraph 1, Article 5 of the Ordinance shall be as follows.

(1) Measures have properly been taken to identify the individual cattle from which the cattle ovaries transported outside the abattoir derive from, until after completion of the post-dressing inspections.

(2) Measures have properly been taken to prevent a loss of cattle ovaries transported outside the abattoir until after completion of the post-dressing inspections.

(3) The facilities at which cattle ovaries transported outside the abattoir are to be preserved constitute a livestock artificial insemination center prescribed in the Domestic Animal Breeding and Reproduction Law (Law No. 209 of 1950), the National Livestock Breeding Center, or an institution engaged in research relating to cattle breeding and reproduction, AND are capable of properly preserving said cattle ovaries until after completion of the post-dressing inspection.

(4) Measures have been taken, by the manager of the abattoir from which cattle ovaries are to be transported, to properly record the name and contact information of the person or entity transporting the cattle ovaries outside the abattoir, the name and contact information of the facilities at which the cattle ovaries are preserved, and other information necessary to ensure a good control system.

(5) Measures have been taken, at the facilities at which cattle ovaries transported outside the abattoir are to be preserved, to properly record the name and contact information of the person or entity transporting the cattle ovaries from the abattoir, the name and contact information of the abattoir from which the cattle ovaries were transported, and other information necessary to ensure a good control system.

3. The standards for giving the permission mentioned in Item (3), Paragraph 1, Article 5 of the Ordinance shall be as follows.

(1) The facilities at which the livestock meat, etc., (meaning the "livestock meat, etc." prescribed in Item (3), Paragraph 1, Article 5 of the Ordinance; the same applies hereinafter) is to be incinerated are capable of properly incinerating livestock meat, etc., pursuant to the provisions of the Waste Disposal and Public Cleansing Law (Law No. 137 of 1970).

(2) Measures have been taken, by the manager of the abattoir from which livestock meat, etc., is to be

transported, to properly record the name and contact information of the person or entity transporting the livestock meat, etc., outside the abattoir, the name and contact information of the facilities at which the livestock meat, etc., is incinerated, and other information necessary to ensure a good control system.

(3) A system has been arranged, by the manager of the abattoir from which livestock meat, etc., is to be transported, to provide a report to the Governor of the prefecture on the completion of incineration of the livestock meat, etc., along with written evidence thereof.

(Diseases for Which Simple Inspections are Performed by Governors of Prefectures)

Article 13. The diseases designated by Ministry of Health, Labour and Welfare Ordinance mentioned in Item (2), Paragraph 2, Article 6 of the Law shall be transmissible spongiform encephalopathy in cattle.

(Scope of Diseases or Abnormalities for Which Inspections are to be Performed)

Article 14. The diseases or abnormalities mentioned in Item (2) or (3), Paragraph 6, Article 14 of the Law shall be as specified in Attached Table 3.

(Matters to be Declared in Written Applications for Inspection)

Article 15. 1. Matters to be declared in the written applications, pursuant to the provisions of Article 7 of the Abattoir Law Enforcement Ordinance, shall be as follows.

(1) Address, name, and date of birth of applicant (for corporations, name, location of principal offices, and name of president)

(2) Date of desired slaughter (in cases of desired dressing of livestock slaughtered pursuant to the provisions of Item (2) or (3), Paragraph 1, Article 13 of the Law, the date of desired dressing)

(3) Species, sex, weight, age (when unknown, the estimated age), characteristics, and production area of the livestock desired to be inspected

(4) Information on medical history of the livestock desired to be inspected

(5) Status of use of animal drugs and the like in the livestock desired to be inspected

(6) In cases of desired dressing of livestock slaughtered pursuant to the provisions of Item (2) or (3), Paragraph 1, Article 13 of the Law, the reason, time and date, and location of slaughter of said livestock in locations other than abattoirs

2. A written application mentioned in Article 7 of the Ordinance for inspections pursuant to the provisions of Paragraphs 2 and 3, Article 14 of the Law, in cases of desired dressing of livestock slaughtered pursuant to the provisions of Item (3), Paragraph 1, Article 13 of the Law, shall be accompanied by a certificate of death or an autopsy report declaring the matters listed in the following items.

(1) Date and time of diagnosis or autopsy

(2) Date and time of death (when unknown, the

estimated date and time)

(3) Species, sex, age (when unknown, estimated age), and characteristics of animal

(4) Name of disease and principal symptoms (in the case of an autopsy report, the state of the carcass instead of principal symptoms)

(5) Address and name of veterinarian performing the diagnosis or autopsy

(Measures Based on Results of Inspections)

Article 16. Measures to be taken pursuant to the provisions of Article 16 of the Law shall be the measures listed in the following items for the respective cases prescribed in the items.

(1) In cases where inspections have been performed pursuant to the provisions of Paragraph 1, Article 14 of the Law and where the livestock are found to have any of the diseases listed in Attached Table 4 or any abnormality: Prohibition of slaughter

(2) In cases where inspections have been performed pursuant to the provisions of Paragraph 2, Article 14 of the Law and where the livestock are found to have any of the diseases listed in Attached Table 4 or any abnormality: Prohibition of dressing

(3) In cases where inspections have been performed pursuant to the provisions of Paragraph 3, Article 14 of the Law and where the livestock are found to have any of the diseases listed in the upper section of Attached Table 5 or any abnormality: Discarding the parts listed in the lower section of Attached Table 5 and other measures necessary to prevent such parts from being used for human consumption

(4) In cases where the livestock are found to have any infectious disease among the diseases listed in the items of Paragraph 6, Article 14 of the Law, or any abnormality, or to be a potential source of disease transmission: Segregation of the livestock; disinfection of meat, viscera, and other parts of livestock; disinfection of the processing room and other locations or properties that have been or may have been contaminated by disease germ; and other measures necessary to prevent the transmission of disease

(Inspection Stamps)

Article 17. When affixing inspection stamps pursuant to the provisions of Article 9 of the Ordinance, the inspection stamps shown in Form No. 1 shall be affixed in accordance with the species of livestock in Attached Table 6.

(Certificates of Inspectors)

Article 18. Certificates which officials concerned must carry pursuant to the provisions of Paragraph 2, Article 17 of the Law shall be those shown in Form No. 2.

Attached Table 1 (Related to Articles 3 and 7)

The head (except the tongue and cheek meat), spinal cord and distal ileum (limited to a 2-meter portion from its junction with the cecum) of cattle; the tonsils, spleen, and small and large intestines (including lymph nodes in these parts) of sheep and goats; and the head (except the tongue, cheek meat and tonsils), spinal cord and placenta of sheep and goats (limited to those over 12 months of age)

Attached Table 2 (Related to Article 6)

Subjects	Numbers of hours
Introduction to public health	4 or more
Laws and regulations relating to livestock slaughtering	4 or more
Livestock anatomy and physiology	2 or more
Livestock internal medicine and pathology	6 or more
Meat hygienics	6 or more
Related laws and regulations	2 or more

Attached Table 3 (Related to Articles 14 and 16)

Q-fever, malignant edema, leukemia, listeriosis, pox diseases, pyaemia, septicemia, uremia, jaundice, edema, tumors, trichinosis and other parasitosis, intoxications, actinomycosis, botryomycosis, fever syndromes, trauma, inflammation, degeneration, atrophy, deformation and injection reactions (only cases with intense responses against biologics), and contamination by lubricating oil or inflammatory products, etc.

Attached Table 4 (Related to Article 16)

Rinderpest, contagious bovine pleuropneumonia, foot and mouth disease, Japanese encephalitis, rabies, vesicular stomatitis, Rift Valley fever, anthrax, hemorrhagic septicemia, brucellosis, tuberculosis, Johne's disease, piroplasmiasis, anaplasmosis, transmissible spongiform encephalopathies, glanders, equine infectious anemia, African horse sickness, hog cholera, African swine fever, swine vesicular disease, bluetongue, Akabane disease, malignant catarrhal fever, Chuzan disease, Lumpy skin disease, bovine viral diarrhea-mucosal disease, infectious bovine rhinotracheitis, bovine leukemia, Aino virus infection, Ibaraki disease, bovine papular stomatitis, bovine ephemeral fever, melioidosis, tetanus, black-leg, leptospirosis, salmonellosis, bovine campylobacteriosis, trypanosomiasis, trichomoniasis, neosporosis, hypodermosis, Nipah virus infection, equine influenza, equine viral arteritis, equine rhinopneumonitis, equine morbillivirus pneumonia, horse pox, tularemia, contagious equine metritis, equine paratyphoid, epizootic lymphangitis, peste de petits ruminants, contagious ecthyma, Nairobi sheep disease, sheep pox, Maedi-visna, contagious agalactia, enzootic ovine abortion, toxoplasmosis, mange, goat pox, caprine arthritis-encephalomyelitis, contagious caprine pleuropneumonia, Aujeszky's disease, transmissible gastroenteritis, porcine

enterovirus encephalomyelitis, porcine reproductive and respiratory syndrome, vesicular exanthema of swine, porcine epidemic diarrhea, atrophic rhinitis, swine erysipelas, swine dysentery, Q-fever, malignant edema, listeriosis, pox diseases, pyaemia, septicemia, uremia, jaundice (severe cases only), edema (severe cases only), tumors (only cases occurring in multiple sites in meat, organs, bones or lymph nodes), trichinosis, cysticercosis (*C. cellulosae*), cysticercosis (*C. bovis*) (systemically affected cases only), intoxications (only cases that may be of harm to people), fever syndromes (only cases with severe fever) and injection reactions (only cases with intense responses against biologics), and contamination by lubricating oil or inflammatory products, etc., (systemically contaminated cases only)

Attached Table 5 (Related to Article 16)

Diseases	Parts
Diseases given in Attached Table 4	All meat, viscera, and other sections of said animals
Jaundice (only cases where lesions are localized in part of meat or viscera)	Such lesions and blood
Edema (only cases where lesions are localized in part of meat or organs)	Such lesions and blood
Tumors (only cases where lesions are localized in part of meat, viscera, bones, or lymphnodes)	Such lesions and blood
Parasitosis (excluding trichinosis, cysticercosis (<i>C. cellulosae</i>), and cysticercosis (<i>C. bovis</i> ; systemically affected cases only))	Parts from which parasites cannot be separated and, in cases of sarcocystis, blood
Actinomycosis	Such lesions and blood
Botryomycosis	Such lesions and blood
Trauma	Such lesions
Inflammation	Such lesions, parts contaminated by inflammation products, plus blood for polypurulent inflammation
Degeneration	Such degenerated parts
Atrophy	Such atrophied parts
Deformation	Such extremely deformed parts
Abnormal organ shapes, size, hardness, colors or odors (only cases where abnormalities are localized in part of the organs)	Organs with such abnormal parts
Contamination by lubricating oil or inflammatory products, etc. (excluding systemically contaminated cases)	Meat, organs, bones and hide with such contamination

Attached Table 6 (Related to Article 17)

Species of livestock	Part to be stamped
Cattle, horses, sheep and goats	(Meat) Back (outside) (Viscera) Any of heart, lungs, liver, stomach, and intestines (Hide) Tail-head (inside) <i>Provided, However</i> , That not being served for food is sure, stamping is not required.
Swine	(Meat) Back (outside) <i>Provided, However</i> , That in cases of processing by the boil-scalding method, stamp the hide of these locations. (Viscera) Any of heart, lungs, liver, stomach, and intestines (Hide) Tail-head (inside) <i>Provided, However</i> , That in cases of processing by the scalding method, or not being served for food is sure, stamping is not required.

Form No. 1 (Related to Article 17)

Type of animal	Form	Remarks
Cattle	Inspected/ Prefecture Name/ Abattoir Serial No.	Shall be an ellipsoid of horizontal diameter 6.6 centimeters and vertical diameter 4 centimeters
Horses	Inspected/ Prefecture Name/ Abattoir Serial No.	Shall be a rectangle of 4 centimeters horizontally and 5 centimeters vertically
Swine	Inspected/ Prefecture Name/ Abattoir Serial No.	Shall be a circle of 4 centimeters diameter
Sheep and goats	Inspected/ Prefecture Name/ Abattoir Serial No.	Shall be a hexagon inscribed in a circle of 4 centimeters diameter

Note: Abattoir serial numbers shall follow the designations of the governors of prefectures or mayors of cities with health centers.

Form No. 2 (Related to Article 18)
(Front)

12 cm	<div style="display: flex; align-items: center; justify-content: center;"> <div style="border: 1px dashed black; width: 150px; height: 100px; display: flex; align-items: center; justify-content: center; margin-right: 20px;"> <div style="display: flex; gap: 10px;"> <div style="width: 10px; height: 10px; background-color: black; border-radius: 50%;"></div> <div style="width: 10px; height: 10px; background-color: black; border-radius: 50%;"></div> <div style="width: 10px; height: 10px; background-color: black; border-radius: 50%;"></div> </div> <div style="text-align: center;">Adhere photo</div> </div> <div style="border: 1px solid black; padding: 5px; text-align: center;"> Seal of governors of prefectures (or mayors of cities) </div> </div>
	No. Division Job name Name Date of birth Certificate of abattoir inspector Issued date (valid for one year)

8 cm